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LAZARUS REPORT

Introduction

The former Lazarus Group envisions the establishment of a goal-orientated, comprehensive, integrated and, multifaceted research program. This program could develop a therapeutic intervention capable of saving the lives of gravely wounded combat casualty dying of exsanguinating hemorrhage. The main thrust of the investigational work would be to find a means of inducing temporary tolerance to the global ischemia that develops during severe hypoperfusion with impending cardiac arrest. The contents of this report arise from the deliberations of the Lazarus Group in Feb and Aug 1995. The goal of the proposed research would be to make possible what is known in science fiction literature as "suspended animation." Even though the term "suspended animation" describes what we wish to be able to achieve, we prefer not to use this term because of its somewhat unscientific and fanciful connotation.

Rationale

The rationale for this report arises from the following facts and observations:

- US Army combat mortality has shown little change since the middle of WW II.
- 90% of total combat mortality occurs on the battlefield before the casualty enters the medical system.
- For the above reason, technological developments and therapeutic strategies that focus on hospital level care cannot, by themselves, significantly decrease combat mortality.
- One-half of the casualties who die on the battlefield die of exsanguinating hemorrhage leading to severe hypoperfusion and culminating in cardiac arrest.
- 80% of these casualties have a vascular injury in the torso as the site of hemorrhage.
- There is no effective first aid for such injuries, and death occurs too rapidly for conventional surgical care to be effective.
- About one-third of these casualties have injuries that surgical care could cure.
- Although research efforts have been directed toward developing interventions designed to "stabilize" bleeding casualties until surgery is possible, the goal of such therapy has been to maintain cardiac output and peripheral perfusion.
- Appendix 1 contains anatomical and clinical data for twenty casualties found in the WDMET database. These cases are representative of the population who die from exsanguination but are surgically salvageable.
- These data indicate that a therapeutic strategy designed to"stabilize" the bleeding casualty by maintaining a functioning circulation given uncontrolled hemorrhage must be considered highly dubious.
- The alternative approach is to accept the inevitability of severe hypoperfusion and cardiac arrest, and to develop a therapeutic intervention that ensues that global ischemia does not result in.
- Irreversible cellular injury that precludes recovery of function.

The primary purpose of the research - to reduce combatmortality - may seem at variance with the medical department's traditional focus of "conserving the fighting strength." The traditional mission is predicated upon World War-like conflicts with vast numbers of casualties. More probably late 20th century warfighting scenarios for the US Army fall into the category of peace making or enforcing operations which are characterized by a limited number of casualties. Because of the high

visibility of such operations and the frequent lack of national unity concerning American involvement, there is a high "political cost" attached to casualties and especially dead casualties. It has never been more incumbent upon the medical department to do everything necessary to minimize deaths since it is no exaggeration to say that for some the word "casualty" has become synonymous with mortality. Nothing in the rationale for the Lazarus Group's program should be construed as minimizing the importance of conventional field first aid or alternative approaches to casualty reduction such as better small unit tactics and the use of more effective protective equipment. Nevertheless, it is our contention that barring some revolutionary development in ballistic protective material, the only substantial casualty population in which a decrease in combat casualty mortality is possible is in the group that we here define -soldiers who die on the battlefield from exsanguinating hemorrhage but for whom there is no effective first aid.

Illustrative Example

We foresee the following sequence of events.

- The soldier sustains a penetrating injury to the torso and soon afterwards appears to be in shock.
- The medic's actions are guided by data derived from biomedical sensors which are a component of the casualty's personal equipment, telementoring with higher echelon medical personnel and, use of computerized decision algorithms.
- The therapeutic intervention, which will probably be pharmacological in nature (although we do not rule out a physical device used in conjunction with drugs), is performed by the medic.
- The casualty, now probably without effective cardia-pulmonary function, is evacuated in an LSTAT to a conventional surgical hospital or a mobile ICU-OR.
- The casualty is placed on hypothermic cardiopulmonary bypass while the bleeding site is surgically exposed and hemostasis/homeostasis is obtained.
- Cardia-pulmonary-cerebral resuscitation occurs.
- For planning purposes, the elapsed time from cardiac arrest to the surgical phase will be about one hour.

Overview of Research Problems

The research program would address three major problems.

- What will be the nature of the therapeutic intervention (the essential feature will be the protection of ischemia intolerant organs such as the brain and heart and possibly the abdominal viscera)?
- How to recognize that the therapeutic intervention will be appropriate treatment for a given casualties?
- How to actually give or implement the therapeutic intervention?

Finding strategies for protecting the brain and heart will no doubt be the most complex as well as the scientifically interesting part of the research program. Nevertheless, the importance of recognizing casualties for which the treatment strategy is indicated and of actually administering the

treatment should not be underestimated. Even the most brilliant solution to the problem of protecting the heart and brain will be useless if no method can be found for conveying medications to these organs in an extreme state of cardiopulmonary dysfunction can be devised. Thus it is inapparent that only a research program that is comprehensive and integrated will have an optimal chance of success.

Scientific Aspects

This preproposal is not the appropriate forum for an in-depth review of the pathophysiology of cerebral and cardiac ischemic injury. However, certain observations are appropriate here since they are indicative of our approach toward achieving the goal of ischemia tolerance. The primary defect that exists during ischemia is an imbalance between cellular high energy phosphate needs and supply. The initial manifestation of the energy failure is an inability to maintain normal membrane function. In neuroexcitatory tissue, cells depolarize as voltage gated sodium channels open. Associated with the influx of sodium is an influx of calcium. A vast cascade of deleterious processes - many of which are caused by excess intracellular calcium - follows ischemic depolarization. In the brain, calcium overload is augmented by the effect of excitotoxic amino acids. Further damage - considered under the rubric of reperfusion injury - arising from oxygen derived free radicals, cytokines and other mediators can be expected during resuscitation when bloodflow is restored to ischemic organs. The intolerance of the brain to ischemia is especially marked and is the major obstacle to be overcome (irreversible injury usually develops from as little as 10 minutes of normothermic ischemia). Even brief periods of ischemia(minutes) may cause delayed (days) death of certain unusually sensitive neurons. The cause is not known but may involve own regulation of synthesis of enzymatic proteins required for normal mitochondrial function. Thus delayed cellular death may also be a manifestation of energy failure but one occurring long after the initial ischemic insult.

The injury process is a continuum. However, it is conceptually useful to view it as occurring in two phases: that associated with the initial energy imbalance, and that caused by reperfusion. Reperfusion injury is an increasingly recognized and understood phenomenon that has been widely and extensively studied, both experimentally and clinically, for over a decade. However, the initial phase of injury has been less well studied. It will form the main thrust of our basic science research effort. It is possible that by preventing ischemic membrane dysfunction from occurring during the initial phase, reperfusion injury and other abnormalities such as delayed cell death during resuscitation will be minimized.

It is obvious that the cellular energy imbalance that exists during profound shock and cardiac arrest can be improved by either increasing the supply of high energy phosphates or by decreasing their consumption. Although inducing cells to use more adventitious metabolic pathways may be possible, a more useful approach will be to down regulate or to actually arrest metabolism. Crucial to understanding how to do this is to know what metabolic processes remain active in the ischemic state. Ion pumping associated with restoring normal transmembrane ion gradients following depolarization is probably the predominant consumer of high energy phosphates in neuroexcitatory tissue.

A conceptually attractive approach to minimizing energy consumption due to ion pumping

is to hyperpolarize cellmembranes either by opening ATP-dependent potassium channels or by blocking voltage gated sodium channels. The former approach using potassium channel openers has recently been shown to result in a remarkable degree of protection during normothermic ischemia in a porcine heart preparation. Furthermore, perhaps hyperpolarizing of neuroexcitatory tissue in conjunction with blockade of NMDA-glutamate receptors might minimize influx of extracellular calcium.

We recognize that hyperpolarizing neuroexcitatory tissue will cause cessation of function which, in the heart, will mean cardiac arrest. It may seem paradoxical that we accept cardiac arrest a part of a therapeutic strategy designed to treat cardiac arrest, but there is a fundamental difference between cardiac arrest that follows exsanguination and what we propose. Our strategy is an extrapolation from the several decades of experience with myocardial protection in cardiac surgery which show clearly that for metabolic arrest to be protective, it must be induced before the heart becomes ischemic. Allowing ischemia intolerant organs to ceases function because of ischemia maximizes injury. Ideally, we wish to bring about metabolic arrest of the brain and heart before exsanguination cardiac arrest occurs.

It is for this reason that recognition of which casualties will be appropriate candidates for the approach to combat casualty care described here is of great importance. Not only will the logistic burden (intensity of ICU care, etc.) of caring for such casualties be high, but the therapeutic margin will be low, since any pharmacological intervention that is capable of causing the degree of metabolic down regulation required to be protective and to preserve ischemia intolerant organs will almost certainly result in cardiac arrest. It will be essential to develop algorithms that have a high degree of sensitivity and specificity.

The following scenario illustrates some of the characteristics of

- the triage methodology needed for casualty recognition:
- the casualty has a penetrating chest or abdominal wound
- a head injury is not present
- the casualty exhibits signs and symptoms of shock and appears to be deteriorating
- the personal status monitor gives evidence of cardiovascular collapse
- telementoring and use of artificial intelligence result in the decision to intervene

The actual mechanics of how to give the intervention are in need of study. Since the casualty's own circulatory system is certain tone inadequate to assure reliable and rapid dissemination of pharmacological agents, some means of assistance will be needed. Closed chest cardiac massage is not likely to be effective in the presence of a vascular injury but an alternative approach is not obvious. The means of administration is also in need of study: intravascular, intra cardiac, or transtracheal injection are possible approaches that will need to be studied for both effectiveness and field feasibility.

Structure of the Research Organization

The research program has four main components (Appendix 2):

- A basic science group, whose knowledge of the biochemistry, physiology, biophysics, and

pharmacology of cerebral and cardiac ischemic injury will lead to testable hypotheses concerning therapeutic interventions

- A therapeutic trials group that will use reproducible and realistic animal models to test the therapeutic interventions proposed by the basic science group and will refine the existing approaches to cardiac-pulmonary-cerebral resuscitation
- A clinical applications group that will develop field applicable strategies for recognizing treatable casualties and implementation of the therapeutic intervention.
- A scientific advisory committee that will provide a mechanism for joint planning, assure integration of the program, and a mechanism for obtaining expert knowledge not otherwise available in the military medical research commands.

An overview of each of the components follows .

Basic Science Group

- Will answer basic scientific question related to tolerance to ischemia such as but not limited to:

  1. lessons from species that are tolerant to ischemia
  2. energy balance in cells under normal and ischemic condition
  3. determine which metabolic processes can be arrested and how to do it
  4. whether the energy deficiency can be made up by using more energetically efficient pathways

- Will propose paradigms that can be used to develop strategies for inducing a state of ischemia tolerance

- Will use an ex vivo brain slice model and other neurophysiological preparations to test the hypothesis that use of ATP-dependent potassium channel openers, by preventing ischemic depolarization, confers protection against ischemic injury. In addition the effect of super high concentration of inhalational anesthetic agents on membrane function will be demonstrated.

- Will use intact animal models to test the hypothesis that blockage of voltage gated sodium channels will confer protection on neuroexcitatory tissue during ischemia.

Experimental Therapeutic Trials Group

- Will carry out therapeutic trials (and monitoring of mechanism - specific pathophysiologic changes) in experimental animals of pharmacological agents and physical interventions, that are found by the Basic Science Group or others to be promising
- Will use their established, reproducible, large animal outcome models, as for example:

1. Exsanguination cardiac arrest and resuscitation with normothermia throughout - to evaluate pharmacologic protective agents, with arrest times of 10-20 minutes. Brief cardiopulmonary bypass will be used as a research tool.
2. Exsanguination with normothermia, followed by induction of hypothermic circulatory arrest.

- Will simulate the field scenario of inducing protection of the organism during exsanguination with drugs and mild to moderate spontaneous cooling by exposure, followed by portable bypass - induced profound hypothermic protection in mobile ICU-or surgical hospital,
- Will optimize protective and resuscitative fluids.
- Will refine the use of hypothermic cardiopulmonary bypass as the essential component of resuscitation at the hospital level.
- Will optimize cardia-pulmonary- cerebral resuscitation for preventing and treating reperfusion injury and complications of rewarming.
- Will collaborate with Dr. Halperin on developing field applicable methods for medicating the pulseless organism.

Clinical Applications Group

- Will determine whether the existing civilian trauma databases contain a subpopulation of casualties who experience prehospital death but who have injuries which, although refractory to first aid, are surgically correctable. Such efforts could be integrated with related work on LSTAT, TCIMS, and for developing indications for telepresence surgery
- On the basis of the above work, will develop a field-applicable algorithm which can be used to identify appropriate casualties for intervention
- Will explore the design of a Phase One clinical study using trauma victims who arrest on admission to the University of Maryland Shock Trauma Center of interventions found to be successful by the therapeutic trials groups

- Will investigate field-applicable methods for administering possible pharmacological agents

1. methods for introducing the agents into the circulation
2. methods for distributing the agents to the tissues under very low flow or no flow situations

- Will investigate the feasibility of field applicable methods for maintaining blood flow to vital organs

Scientific Advisory Committee

- Will serve as the administrative interface between the funding agencies and the research groups
- Will provide scientific and financial oversight
- Will organize meetings to discuss progress and the need for possible changes in research directions and to provide independent scientific critique
- Will develop a mechanism for obtaining rapid answers to scientific questions that may arise in the course of the investigation

Conclusion

It is apparent that the products of a successful research program will not only benefit combat casualty care but will find considerable application in civilian trauma management as well as in the prevention and treatment of such diverse conditions as stroke and myocardial infarction and sudden cardiac death.

Appendix 1

Representative casualties who were killed in action but who had surgical correctable injuries that caused death by exsanguination

1. Casualty appeared lifeless immediately after sustaining obliquely perforating thoracoabdominal gunshot wound; lung and liver injured; a total of 1 liter of blood was found in the chest and abdomen.

2. Casualty appeared lifeless immediately after sustaining thoracoabdominal gunshot wound which lacerated liver and left lung and transected descending aorta; 1100 ml of blood found in left hemithorax.

3. Casualty appeared lifeless immediately after being struck by bullet which entered neck and exited through flank; laceration of left subclavian artery; 800 ml of blood found in left hemithorax.

4. Casualty appeared lifeless within a minute after being struck by multiple fragments in the right hemithorax; 1300 ml of blood found at autopsy.

5. Casualty appeared lifeless within 2 minutes after sustaining transverse, perforating thoracoabdominal gunshot wound with injury of the right lung, liver and kidney; 1100 ml of blood found within right hemithorax, 1500 ml of blood found in abdomen.

6. Casualty appeared lifeless within several minutes of being struck in abdomen by a bullet; injuries to the small bowel, stomach and mesocolon; 2000 ml of blood found in abdomen.

7. Casualty appeared lifeless within several minutes after sustaining posterior-anterior perforating gunshot wound of the upper chest; laceration of the innominate artery; 1700 ml blood found in right hemithorax.

8. Casualty appeared lifeless three minutes after being struck by obliquely perforating gunshot wound of chest; laceration of both lungs and fracture of T-2; total of 1500 ml of blood found in both hemothorax.

9. Casualty appeared lifeless after 5 minutes after sustaining multiple fragment wounds of chest and extremities; 1400 ml of blood found in both hemithoraces

10. Casualty appeared lifeless within five minutes after sustaining multiple fragment wounds of the chest and abdomen; injuries of the lung, liver, small bowel and colon: 1500 ml of blood found in chest and abdomen.

11. Casualty appeared lifeless after about 10 minutes; through and through gunshot wound of abdomen; bullet entered right flank and exited under left costal margin; celiac axis and superior mesenteric vein transected; 1750 ml of blood found in abdominal cavity.

12. Casualty appeared lifeless after 10-15 min after being wounded: bullet entered laterally on the

right side of the abdomen and passed out of left hip; autopsy showed transection of right internal iliac artery, sigmoid colon and left femoral artery, multiple perforation of small bowel; 1750 ml of blood in abdominal cavity.

13. Casualty appeared lifeless after 10-15 minutes; perforating gunshot wound of abdomen with lacerations of liver, duodenum and kidney; 1300 ml of blood in abdomen.

14. Casualty appeared lifeless 15 minutes after sustaining perforating gunshot wound of chest; bullet entered right anterior axillary line and exited to the left of the spine above the iliac crest; laceration of lung, fracture of T12; severed spinal cord; 2000ml of blood found in right hemithorax.

15. Casualty appeared lifeless 15 minutes after sustaining two gunshot wounds of the upper chest; one bullet after fracturing T 4-5, lacerated the trachea, esophagus and one lung; second bullet passed through chest into abdomen where it injured the liver, kidney, pancreas; 250 ml of blood found in abdomen.

16. Casualty appeared lifeless 15-20 min after sustaining two perforating gūn shot wounds of left side of body; one passed through the arm and entered the chest where it fractured four ribs -no description of hemo-pneumothorax; second bullet fractured T10 and severed the spinal cord at that location.

17. Casualty appeared lifeless 20-25 minutes after bullet entered right flank and passed through liver, diaphragm and all lobes of right lung, several severely fractured ribs at wound of exit; 650 ml of blood found in chest and abdomen.

18. Casualty appeared lifeless 50 minutes after gunshot wound of chest with fractures of ribs 7,8, 9; small hemothorax (250ml); massive "traumatic atelectasis" of lung (pulmonary contusion?);casualty became unresponsive 2 minutes after being wounded and after he stated "I think I've got a punctured lung."

19. Casualty appeared lifeless within one hour of sustaining a perforating gunshot wound which entered right shoulder and exited in lower abdomen; lacerations of right lung, diaphragm, liver and kidney; a total of 1600 ml of blood was found in the chest and abdomen.

ADVISORY COUNCIL ON PHARMACOLOGICAL STABILIZATION

HEALTH GOVERNMENT RELATIONS AND CONSULTING

Gray & Associates

Friday, August 18, 1995

Ritz Carlton

Pentagon City, Virginia

C O N T E N T S

P R O C E E D I N G S

COLONEL BELLAMY: Thank you all for coming. On behalf of General Zajtchuk, I'd like to welcome you to this meeting, which probably is the last meeting of the Lazarus Group in its present format. We have, I think, interesting developments which Commander Yaffe, representing the Navy, will tell us about in the second part of our program.

Let me say that General Zajtchuk is not here because, being a general officer, he's extremely intelligent and was smart enough to get out of this horrible place and go to Maine, which we should all do.

COLONEL BELLAMY: The weather actually does have some indications to what we're going to talk about, though, because as the enthusiasts for hypothermia and hibernation should realize, the United States Army insists upon fighting all its wars in extremely hot places. So there are environmental limitations placed upon using hypothermia and hibernation.

There are a number of new people here, and what I'd like to do is very briefly give an introduction to what the rationale for this group is. Some of you who were here before have already seen this material. The background of the problem is that historically, during this century, about 20 to 25 percent of wounded United States Army soldiers are killed in action. The official definition of being killed in action is a casualty who die on the battlefield before any effective care is given.

This proportion, believe it or not, has really not changed significantly during this century. There's been a fall in the percentage of casualties who die at the hospital level, from about 8 percent in World War I down to about 3 percent more recently. But the fact of the matter is death on the battlefield has not changed during this century, even with very rapid evacuation and probably better medical training and obviously better surgical and critical care.

Since the beginning of World War II, 90 percent of the total United States Army combat mortality falls into the category, killed in action. Death is caused by exsanguination due to a penetrating missile in about one-half of the soldiers killed in action. The site of the injury is in the chest or abdomen, in 80 percent. I'm sure Dr. Champion would agree there really is no effective first aid for such casualties. For one thing, they die very rapidly, and, in fact, the data we have show that about 70 to 80 percent of the casualties who fall into this category are clinically dead within 5 or 10 minutes, and in many cases even less than that. They die very rapidly. There is no time to apply any existing medical treatment.

Interestingly, autopsies of these casualties indicate that about one-third have injuries in which surgical

care could, in all probability, easily correct the injury. These are injuries such as a lacerated iliac artery, a lacerated mesenteric artery, or even perforatings of the heart with small wounds of entrance of exit.

Of course, the problem is such casualties are clinically dead before they can even be evacuated, even with very rapid evacuation times on the order of less than one hour.

So the rationale for organizing this meeting was to speculate as to what kind of therapeutic intervention could be developed, if any at all, that would allow survival, in an apparently lifeless state but with viable organs, until such casualties could be evacuated to a surgical hospital. I'm talking about time between injury and the arrival at a surgical hospital of an hour or so.

What we need to do is develop a treatment which would induce, as I say, a temporary state of tolerance to the systemic ischemia that occurs with cardiac arrest and exsanguination.

Now, I purposely excluded other injuries such as those involving the brain. Although injuries of the brain are a very important cause of death - about a third of the people who are killed in action die of brain injuries - they are catastrophic injuries beyond any possible treatment that I can imagine. In fact, the overall mortality with the brain injury casualty in the database we have from Vietnam is that a missile striking the head reaching the periosteum of the skull, the probability of being fatally injured is 78 out of 100.

There's also another group that is a major cause of death, casualties who sustain mutilating injuries from explosive devices where there's no possible medical treatment.

So we're dealing with a selected population: Those who die of exsanguination of such casualties, 80 percent have wounds in their abdomen or chest, and about a third of them have injuries which at autopsy appear to be easily correctable.

I think that there are very important logistical problems associated with using such an intervention. First of all, what are the indications. How do you decide a person would be, in fact, a candidate for this treatment? It's also important to try to organize what goes on in terms of phases, which I'll talk about. Obviously the major thrust of this meeting is what are the pathophysiological derangements that occur during ischemic injury? What organs are most likely to be affected? And, of course, the people here obviously realize we have four specialists on brain ischemia to talk about it, so clearly, the organ of greatest importance will be the brain. But we also are concerned about the heart and other organs to some extent. Ultimately, what can be done to prevent or reverse the pathophysiological derangements which afflict these organs?

Let me briefly talk about the logistics of intervention. Clearly, to apply any kind of therapy, we have to have people there to do it; that involves prompt arrival of medical personnel at the site of the casualty.

Interestingly, the data from Vietnam indicate that it may not be all that difficult because in the Vietnam War, the median time between wounding and the arrival of the first health care provider, the medic, was only four minutes. So that does give a little bit of time to take care of at least some of the people who fall in the death from exsanguination category.

An additional problem. Battlefield conditions may or may not allow a therapeutic intervention to be made. I would estimate that probably half the time nothing can be done because the situation on the battlefield precludes anybody from getting to the casualty without being killed themselves. But, still, there's a huge potential for life saving interventions in the remaining one-half.

The availability of a simple and easily applied intervention is a necessary prerequisite. Complicated equipment will not be feasible. One can imagine some sort of therapeutic cocktail, with infusion of the solution.

There will also have to be some means of assuring systemic dissemination of these active agents. Obviously, the person that is on the verge of cardiac arrest, in fact, and will have a cardiac arrest caused by giving these agents does not have an effective circulation. How will the therapeutic material be disseminated to those organs where it has to be present to have any effect? Perhaps one could use closed-chest cardiopulmonary resuscitation to disseminate an agent. It seems to me these are important questions which cannot be considered divorced from the basic problem of managing the ischemic problems or preventing them.

Once cardiac arrest has occured, the availability of rapid transportation in a medically sophisticated vehicle becomes necessary. The question is actually being addressed by the Advanced Research Projects Agency. They have a number of contracts, including one with the Army's facility at the Walter Reed Army Institute of Research to develop just such a device that would allow the transportation of a casualty who is in a state of pharmacologically induced temporary tolerate to ischemia. The availability of sophisticated resuscitation and surgery in critical care facilities is also necessary. Fortunately, many of the problems in these areas have already been solved or are being addressed by civilian groups. The real problem becomes identifying the casualty population.

Now, let's talk about indications. Whenever I give a talk about this subject, people always look rather incredulous because they can't imagine that a medic, who is the least trained individual in the Army medical department will be making what is the most fundamentally important clinical decision you can imagine. So how is that to be done? You can't depend upon this young man or young woman making a decision under the awful circumstances existing on

the battlefield. So how can it be done? We have to have some guidelines. At least, we have to think about what would be the indications for this sort of intervention.

First of all, we're talking a missile wound of the trunk in the absence of a direct brain injury, clinical evidence of shock and pulselessness. Now, this should not be too difficult to determine, but the real saver in this situation, turns out to be work being carried out right now by the Advanced Research Projects Agency with a variety of the advanced technologies involving electronic communications.

One would be the personal status monitor, which Dr. Jenkins has promised and he will tell us about at lunchtime. This is a device for measuring on a real-time basis, routinely measured vital signs. It could well be that you could use this device, at least to give concrete evidence to a medic as to what is really wrong with the casualty. Certainly, heart rate can be monitored and other indices, perhaps transcutaneous pH and oxygen saturation and so on. So this may well allow us to go well beyond simple clinical signs in helping to make the determination that a given casualty is a candidate for this putative therapy.

Finally, what also is being done by ARPA is real-time decisionmaking based upon telementoring and teleconsultation. The medic is instrumented with devices which will allow him to communicate with a more senior physicianat some position in the rear. It is possible that the combination of the personal status monitor, with decision making using telementoring and teleconsultation will take away one of these, will make possible a solution to the very serious problem of who decides what to do and when.

Now, let's talk about problems which are more in line with the purpose of today's meeting. My personal view is that it is possible to categorize the phases of intervention as follows: First of all, the injury phase, immediately after the injury, exsanguination and shock. The casualty then undergoes cardiopulmonary arrest either because of exsanguination or because of the therapy itself. Obviously, as you'll see, one of the keys to this, in my view, is that you have to have some way of downregulating the metabolic rate prior to cardiac arrest. The second phasewill be of cardiopulmonary arrest with ongoing ischemic injury.

Finally, the casualties will be resuscitated, this phase involving surgery and critical care. I would say that probably the most important problem, other than the surgery care of the injury, will be preventing the development of reperfusion injuries once the circulation that is reestablished. It is my personal view to look at what needs to be done in these three phases: initial injury, cardiac arrest, and resuscitation.

Now, what about ischemic injury to the brain? Of course, now I'm getting into areas where I really don't know the answer. I really don't know what the questions are, in fact. But apparently during the injury phase itself, during

exsanguination, there is release of stressors and various mediators and exhaustion of high energy phosphates. Clearly, this phase can't be very long. We're talking about a matter of 10 minutes or 15 minutes or less.

Cardiopulmonary arrest occurs, and then, there is a total shortage ultimately of high energy phosphates, which results, initially, in loss of osmotic homeostasis, and, activation of a variety of intracellular processes, which will lead to destruction of membranes and intracellular organelles. If this goes on too long there's no way to resuscitate the individual. But, in fact, if the person does reach the stage where resuscitation surgery and critical care is involved, then we're dealing with a variety of phenomena such as microvascular insufficiency, impaired use of substrates by mitochondria, damage due to the generation of oxygen-derived free radicals and lipid peroxidation. Of course, these are abnormalities the prime manifestations of the reperfusion injury. Finally, the clinical problem will be the development of multiple organ dysfunction, which is the major problem with many people who have severe trauma.

By the way, one of the hopes of this meeting is we have a more informal arrangement so that we can more readily exchange ideas. So, obviously if you have questions, please don't hesitate to ask them.

What organs are most likely to be affected by prolonged circulatory arrest? Normothermic ischemic times, such as 5 minutes are reported for the brain. One might ask why the spinal cord tolerates more like 45 minutes of normothermic ischemia. The heart may tolerate 20 minutes of normothermic ischemia. This is when electronic micrographic evidence of injury first shows up in the mitochondria. Of course, there is a profound derangement of heart function. A few minutes of myocardial ischemia results in problems such as abnormal diastolic relaxationOf course, systolic contraction is impaired. But actual anatomical evidence of injury, I do believe, does not seem to occur until about 20 minutes.

Other organs like the liver and kidney seem to have greater normothermic ischemic progress, maybe an hour, and the gut may be--I don't know how long. Does anybody have any idea?

MAJOR BRUTTIG: For reversible damage, it's probably half an hour. But for irreversible damage, an hour, probably.

COLONEL BELLAMY: An hour, okay. Then skeletal muscle, of course, will tolerate much longer ischemia. We're talking about many hours. So, clearly, the important organs are these two: brain and heart. I guess the problems with liver, kidney, and gut would show up in casualties who, in fact, have reached the stage of resuscitation, surgery, and critical care.

What can be done? I think the common thread here is going to be how to bring about a state of metabolic

arrest. We have to have some way of stopping those processes that consume ATP.

When we get to the resuscitation, surgery, and critical care stage, a lot of putative interventions are possible. Clearly, the first thing we'll do in this phase is to place the casualty on hypothermic cardiopulmonary bypass. At the same time, bleeding will be controlled. One of the difficult problems that may appear will be what to do if, in fact, the casualty has a surgically uncorrectable injury? This is a generic ethical problem that will have to be addressed some time.

Nevertheless, there are all sorts of things you could think about--life support interventions; there's a whole variety, a whole spectrum of things that can be done in ICUs nowadays. And, of course, all these interventions, free radical scavengers and substrate enhancement with Krebcycle intermediates etc., that might reverse the problem with deficient energy utilizations. Nevertheless, the really important problems will be finding what processes consume energy, and what processes can be turned off without destroying the cells.

These are some of the questions I think would have to be answered by a research group dealing with trying to find an approach to metabolic arrest. One question is what is the energy budget of cells? It has been reported that something like 40 to 50 percent of the energy that's consumed by the brain goes into ion pumping. Some of this can be turned off and some of it can't. If it can be turned off then ion channel dysfunction of such a magnitude will occur that normal ionic gradients are lost, calcium comes in, you have activation of all sorts of undesirable processes.

During the resuscitation phase with surgery and critical care metabloism will need to be restarted. It's not a question of turning ATP generation off. It's a question of turning off the processes where it's used without wrecking the cell. So you'd have to find some way of restarting it.

It's my view that the thrust of this organization should address the pathophysiological derangements during the first 5, 10 minutes or 15 minutes of ischemia, rather than the questions which occur hours afterwards during the phase of resuscitation, surgery, and critical care. A lot of work on reperfusion injury and multiple organ dysfunction, is going on in many centers throughout the world.

Let me reiterate what I just said. The major research effort has to go into what is not presently being studied, the derangements that occur very early on, rather than something that happens say a day later.

Questions?

COLONEL BELLAMY: Well, let's begin then. We haven't introduced the members of the group here. Colonel Gray, do you want to do that?

MR. GRAY: Let's all introduce ourselves. I'm Tom Gray from Gray & Associates. We have the privilege of providing administrative support to this group.

One of the things that I should have announced when I gave the administrative announcements was we have Chris from Miller Reporting, and he is recording and transcribing the meeting. He has strategically placed microphones throughout the room to include on the butcher chart paper so we could catch all the remarks.

Now, if we could go around and you could introduce yourselves, let's start with Dr. Bazan.

DR. BAZAN: I'm Dr. Bazan from LSU in New Orleans. I'm the head of the LSU Neural Science Center.

DR. SIESJO: Bo Siesjo from Lund, Sweden, Laboratory for Experimental Brain Research at the University Hospital.

DR. HALLENBECK: I'm John Hallenbeck. I'm the Chief of the Stroke Branch at the NINDS, NIH.

DR. VIRMANI: I'm Dr. Virmani. I'm Chairman of the Department of Cardiovascular Pathology at the Armed Forces Institute.

DR. YAFFE: I'm Dr. Yaffe, representing the Navy Medical R&D Command, specifically the Combat Casualty Care Program.

MAJOR BRUTTIG: I'm Steve Bruttig. I'm Deputy Director of the Combat Casualty Care Program for the Army.

DR. DIGERNESS: I'm Stan Digerness. I'm in the Department of Surgery at the University of Alabama at Birmingham.

DR. CHAMPION: I'm Howard Champion, trauma surgeon, University of Maryland.

DR. LEWIS: David Lewis. I'm an American but I've been working in Sweden for 30 years doing microsurgical research. I'm retired but I was Director of Research at the University Hospital in Sweden.

DR. HOCHACHKA: I'm Peter Hochachka. I work at the University of British Columbia. We have a right to be our own state, but we are forced to be a part of Canada.

DR. SAFAR: Peter Safar, University of Pittsburgh.

DR. BENTLEY: I'm Kim Bentley, and I work at the Naval Medical Research Institute.

MS. GRAY: I'm Dottie Gray, and I'm with Tom Gray & Associates.

DR. TISHERMAN: Sam Tisherman, University of Pittsburgh.

DR. NIELSEN: I'm Dr. Nielsen. I'm a cell biologist at the Medical Research Institute.

DR. FALK: Michael Falk of the Medical Research Institute.

MR. GRAY: We have three others that may be joining us. We expected Dr. Verma from the University of Health Sciences, Dr. Jenkins from ARPA, and Dr. Pierce from Walter Reed Army Institute of Research.

COLONEL BELLAMY: And we hope they come.

MR. GRAY: Yes.

COLONEL BELLAMY: So we'll begin. The first three talks will deal with brain injury, although I think Dr. Safar is going to talk about heart also. Dr. Siesjo will talk about his concepts of brain injuries.

DR. SIESJO: Let me start by saying how delighted I am to be here, and I want to thank firstly Peter Safar for taking the initiative, and Dr. Bellamy and Colonel Gray for handling the invitation.

My background is as follows: I'm an M.D., but I haven't seen a patient for about 35 years. I have a professorship at the Swedish Medical Research Council, and I've been doing research on energy metabolism, brain metabolism for 35 years, and I've worked with ischemia for approximately 25 years.

I have a feeling that this field is moving extremely rapidly now and that we are seeing some fascinating opportunities for treatment just around the corner. And this is what I'm here to tell you about today, and I think we have to revise some of the classical concepts, and Dr. Bellamy showed some of them. I think three or four or five or six months ago, I would not subscribe to many of these concepts, but I think I will be able to suggest today that perhaps we should try to rethink along some of the lines, at least.

I want to start by recalling the finding in the literature which is of, I think, extreme relevance to what we're going to be discussing today. A friend of mine, Peter Hostman in Cologne, had the stubborn idea of looking at 60 minutes of ischemia in the brain and trying to recover the animals, and he could without any problems after a few years recover EEG and integrated functions. But he had difficulties of having the animals survive after the 60 minutes of complete ischemia. But he succeeded in getting a few cats surviving after 60 minutes, and one cat survived for several months.

When they performed the pathology on this cat, it turned out that there were no infarcts in the brain, but there were so-called selective neuro-vulnerability. That I think tells us two things. The first thing is that you can actually avoid the catastrophic events which occur usually

after such long ischemic periods as 60 minutes, and the second thing is that even if you will be able to stop these processes that lead to the catastrophical damage, you are still faced with the problem of selective neuro-vulnerability. So probably we're dealing with at least two major mechanisms leading to the final cell damage after long periods of ischemia.

If we want to look at or examine the mechanisms which are responsible for the selective neuro-vulnerability, it's actually profitable to use relatively short periods of ischemia. If we want to use long periods of ischemia, it's not practical to use, at Peter Hostman, cats or monkeys and try to have them survive after 90 minutes of ischemia. It's more profitable to look at focal ischemia, that is to say, stroke type of lesion, and then look at the recovery after one or two hours of ischemia. That will give us a lot of new information about recovery processes.

So I will start with the first slide, and I have taken for granted that some of you really do not have medical background or a surgical background, so I will try to be elemental. This is a slide from '81 and from Wyllie where he subdivided ischemic damage into two types: necrosis and apoptosis. Ischemia was considered to give rise to necrosis with cells which would swell first and then they would shrink and there would be plasma membrane failure. Apoptosis was considered to be something which occurred when the tissue got rid of redundant cells during development and in a lot of other conditions.

It is now realized that apoptosis actually occurs in ischemia, both in global ischemia with recirculation and in focal ischemia. That is extremely important for all our attempts to try to treat ischemic damage in the future because apoptosis is a process which essentially is program centered. So it would probably involve a DNA program or suicide which all cells may have, but which is normally suppressed, dormant, but which can be activated. And the cell is then dependent on protein and DNA synthesis to kill itself. So we have to ask, what is the signal for that and could we possibly stop this apoptosis process.

Another thing which I just want to remind you of is mitochondria metabolism. It's elementary knowledge that brain cells use glucose. They metabolize that to pyruvate. The pyruvate is oxidized in the mitochondria, and here comes the ATP. The problem with ischemia/hypoxia is that you can't oxidize pyruvates, so this is now reduced to lactate and you get your lactic acidosis.

Mitochondria, they require calcium to regulate some of the enzymes in the mitochondria, but in ischemic conditions, that calcium goes into the cell and then mitochondria make take up after, at least during recirculation, and this may be a major problem. In general, it is now realized that when we talk about reperfusion damage, the mitochondria themselves represent targets for the free radicals and the other things. So, essentially, why

tissues succumb after a long period of ischemia with recirculation could be that you incur mitochondria damage during recirculation.

I think it's important when we discuss ischemia to realize that we have different types of ischemia. One time of ischemia which has been extensively studied in experimental settings is global or forebrain ischemia of the so-called cardiac arrest type. Excuse me, this is not very scientific, but it's just for descriptive purposes.

This ischemia is usually dense and is always transient, and if it's short-lasting, we have the selective neuronal necrosis, and this is very often conspicuous with delay. In some cells, the cells may die off in a couple of hours. In other cells, like a CA1 cell of the hippocampus, it may take two or three days before the cells die, and they can take up function in the period before they succumb. So it's like a bomb which has been planted inside these cells, and it's ticking for a couple of hours or for a few days, and then it flows.

We have to ask a question about it. If we have a more long-lasting ischemia, then we have laminar necrosis or we have infarction, and that's then often perivascular, telling us that now we also get involvement of micro vessels.

The second type of ischemia, which at least the brain people are interested in, would be the focal ischemia of the type which mimic stroke. Then we have a densely ischemia core and a less densely ischemia so-called penumbra zone. This ischemia is often permanent, but we have early or late recirculation. The result is infarction, but with quick reperfusion, we may have selective vulnerability as well. And this is a very useful type of ischemia because we can study hours of ischemia and reperfusion with it and, therefore, really get into the problems which I think are close to your hearts.

What do I mean by dense ischemia? This is an old slide from our laboratory where the blood pressure was reduced in steps and maintained at each step for 20, 25 minutes. And then you observe that catastrophical things happened with a decrease in phospho-creatinine ATP, increases in AMP and ADP, at a very, very narrow interval, with a blood pressure of about 30. And we now know that this corresponds to a fall in blood flow to approximately 20 percent of control. So dense ischemia is something which is 20 percent of control or less than that, at least for the discussion today.

Let's take optimal ischemia or brief duration. This is in the rat, and it's 15 minutes of ischemia, forebrain ischemia, and what we observe is a very rapid fall in phospho-creatinine and in ATP, so this is energy failure. These are observed that it takes only a few minutes, by three, four, five minutes, to deplete the stores in the tissue of ATP. So, Dr. Bellamy, we have very little time, at least for the brain, to try to prevent the fall in ADP. We

have to live with it, I think, that the ATP is essentially zero when you find your victim on the battlefield.

But the important thing now is ATP failure is a trigger to other events which are potentially devastating to cell survival. One is the production of lactate and the fall in extra- and intracellular pH, and another one which I think that Nick Bazan will be discussing is the activation of phopholipases and accumulation of free fatty acids.

Then if you recirculate, you bring back perfusion pressure to an adequate one after 15 minutes, no problems, all these changes will be reversed relatively quickly. But there are problems, and one problem is that the acidosis will persist for some time afterwards, and also you will have elevated levels of free fatty acids for some time when oxygen comes back. And as I will discuss later on, this can start production of lipid metabolites and mediators which are potentially very harmful.

Another thing which comes up like a letter in the mail after ischemia is loss of ion homeostasis with the release of potassium from cells, an influx of calcium, sodium, and chloride, and a fall in extracellular space, indicating now that water is being shifted into the--yes?

COLONEL BELLAMY: Are the indicators on the scale one-minute times?

DR. SIESJO: Yes, sorry.

COLONEL BELLAMY: So within 5 minutes of ischemia, we see the that derangements have already

DR. SIESJO: Yes. With ischemia it takes you approximately one minute in the rat neo-cortex before you'll see the influx of potassium and the influx of calcium, sodium, and chloride. So one or two minutes, that's what we have--

COLONEL BELLAMY: That appears to be synchronous then with the fallen ATP levels.

DR. SIESJO: Actually, the ATP has come down to approximately 30 percent of control when you see this massive loss of ion homeostasis. So you suddenly activate ion conductors there.

DR. HOCHACHKA: These time frames are for a rat brain.

DR. SIESJO: Yes.

DR. HOCHACHKA: How directly applicable are they to humans? You would presume they extend it at least to some degree?

DR. SIESJO: I think you could say that they are related to metabolic rate, and if metabolic rate is lower, as it is in man, it would take probably a longer time to do that.

DR. HOCHACHKA: So for a larger organism like man, you probably could--would you guess maybe for those would become 2-minute intervals, 1-and-a-half minutes?

DR. SIESJO: Yes, probably.

DR. HOCHACHKA: So you would get a bit more time.

DR. SIESJO: A little bit more time, yes. But if you reduce body temperature to 33 degrees or brain temperature to 33, you will only delay the loss of ion homeostasis approximately 30 seconds.

COLONEL BELLAMY: Thirty seconds is all?

DR. SIESJO: Yes. That's for a 4-degree fall in brain temperature. And the rate of influx is the same.

COLONEL BELLAMY: This is intracellular calcium?

DR. SIESJO: Sorry. These are all extracellular reportings. So when the extracellular potassium goes up, it shows you that the cells are losing potassium. When this goes down, it shows that calcium is flowing into the cell.

DR. HOCHACHKA: Is going in, right.

DR. SIESJO: But it can be shown in a much better way. This is from Silver and Oginska, beautiful work, where they prodded more than 1,000 CA1 cells in the rate with a sub-micron calcium electrode and could show that during the ischemia there is an increase from approximately 100 nanomolar to 30 to 60 micromolar in the free cytosolic calcium concentration in these cells. And as you see, it takes very little time before you come up to the maximum calcium level.

But if you recirculate, you also get back to normal in a couple of minutes' time, but then there is one thing which I think we should look at here. Silver and Oginska reported that if they carried on with the recirculation for many hours, they very often saw a slow, gradual, secondary rise in the free cytosolic calcium concentration. I'll come back to that because it's a very important finding.

Let me now discuss with you three different types of ischemia. I would subdivide the first type of ischemia into two, and discuss first ischemia of such brief duration, with such reflow, optimal reflow, that we essentially only get neuronal damage in the selectively vulnerable areas. Then we know that it will restore the cerebral energy state very quickly, and those energy-driven reactions are recovered in less than 5 minutes after recirculation. But metabolic rate is depressed through a long period, and the mitochondria PDH activity is reduced. We also have a reduced synthesis of proteins if you look at overall protein synthesis, but new proteins are potentially synthesized such as heat shock or stress proteins. And you also express messenger RNA for immediate early genes. And if you look at this slide, which is from our own laboratory, in collaboration with the Lenbow School, you see that after ischemia, there is an expression of messenger RNAs up for some of the growth factors. And we

know from results in gerbils that after ischemia of brief duration there is an up-regulation of messenger RNA for SOD. So even an anti-oxidant enzyme is being triggered in the recirculation period.

Why all that? Probably this is the way nature uses to try to boost survival in these animals. What you do is that you cut down metabolic rate; you reduce overall protein synthesis; but you synthesize preferentially such proteins which have a survival background, and you also synthesize proteins which can quench free radicals or do other things. So this is an attempt by the cells probably to survive.

So let's make the assumption now that this site of ischemia is a nonvascular lesion. You only hit the neuron, so that's it. No glial cells are killed and vascular elements are being left intact. And we know that similar neuronal lesions are observed in hypoxia and hypoglycemic coma, so, therefore, we say that probably this is a reasonable assumption.

But in all these conditions, we have a loss of ion homeostasis with influx of calcium into cells. So let's make the assumption now that calcium is triggering the delayed cell death of these cells. When we say cell, we have to be interested in the mechanisms, and I'm sorry for the complexity of this slide, but it just tells you that what a rise of calcium will do is to activate lipolysis, alter protein phosphorylation, activate proteolysis and lead to desegregation of microtubuli, and not shown here is that it will also lead to an activation of endonucleases in the nuclei.

Lipolysis can be bad, as probably Nick Bazan will be mentioned, but it can be bad because it gives rise to the production of free fatty acids, lysophospholipids, to PAF, to leucotrienes. Protein phosphorylation can lead to changes in receptor and ion channel activity to an altered gene expression and affect protein synthesis. Possibly it can also activate the suicide program.

Then, of course, proteolysis and desegregation of microtubuli can lead to a breakdown of the cytoskeleton and lead to inhibition of axonal transport on blooding. But I want also to point out that there is accompaniment in the rise in calcium and the production of free radicals because calcium would lead to activation of some enzymes like nitric oxide synthase and to an enzyme conversion XDH-XO, et cetera, and, of course, you activate also the phospholipids which would give rise to free fatty acids, which then will be converted to the cytoxamase and lipoxamase pathway. So you do get a cascade of events leading to free radical production.

This is just for an overview, and I want to immediately ask the question: If calcium is entering the cell during brief periods of ischemia, by what pathways will calcium enter? And is it possible to use blockers to prevent the influx of calcium into cells? Is that something that Dr. Bellamy and all of you would be interested in pursuing? And

I would say probably this is, and I will tell you why. This is an old slide, so it gives you only two types of voltage-sensitive calcium channels, presynaptical, P and N. We have few nalars. I think John Hallenbeck will mention that. And then post-synaptically we have L and T, and they may also be pre-synaptical. But the point is that we have one, two, three, four, five--at least five types of voltage-sensitive calcium channels.

Then when glutamate is being released, it acts on two types of inotropic receptors, one activating the AMPA receptor, which gates the channel which is permeable to monovated cations, and the other one activating the NMDA receptor, whose channel has permeated calcium. But this permeability is normally blocked by magnesium in physiological concentrations.

But look here now what happens if glutamate pours out presynaptically, you activate both receptors. When you activate the AMPA receptor, in comes sodium. When sodium comes in, the membrane will depolarize. Out pops magnesium, and now calcium can go in through this channel. But depolarization would allow calcium to go in through all the voltage-sensitive calcium terminals and usual oxyo-reversion of the sodium calcium extincture. So we may have six, seven, eight, nine different channels by which calcium are going into the cell. How could you block all of those? Specific pharmacology is not very useful, and unspecific pharmacology we don't have yet.

Then there is another problem. We know that in many cells there is an abundance of NMDA receptors, and therefore, calcium influx through that channel gated by glutamate receptors could be particularly important say in the CA1 cells.

Then we should look again at Silver and Oginska. Would a calcium electrode in a CA1 cell and barbiturate anesthesia, ischemia, up goes the free cytosolic calcium concentration to high levels. What they did now was to give ketamine or MK-81 which would block the NMDA receptor's gated channel, and then they found a sluggish increase in the free cytosolic concentration. So you could say, aha, here is the solution. We'll just pour in NMDA receptors and we retard the influx of the free cytosolic concentration.

But here is the problem. Many good groups have now shown that if you use NMDA receptor antagonists, whether they are competitive or non-competitive, you cannot ameliorate the damage due to dense forebrain or to global ischemia, in spite of the fact that you can retard the influx of calcium. But you can use an AMPA receptor antagonist and you can ameliorate the damage. So you have to explain that. You also have to explain why both NMDA and AMPA receptor blockers are good with stroke lesions but not with forebrain ischemia lesions. So how on earth could we explain that?

Many years ago an American anesthesiologist, when he worked in our lab, produced results with 10 minutes of forebrain ischemia, with measurements of the calcium

concentration, total calcium concentration in dorsal hippocampus, and found that it took at least 24 hours for the calcium content to start rising in the tissue. And we have checked that with techniques, histological sections, the same result. But the interesting thing was that the accumulation of calcium here seemed to precede signs of morphological death in the cells. So what we did was to put out the hypothesis where we said that if we have transient ischemia, we get a sustained perturbational membrane function, and perhaps with an increased calcium cycling across the membranes. But the point is that we now have a gradual rise in the free cytosolic calcium concentration, and when calcium exceeds the set point for net uptake of calcium by the mitochondria, we get calcium overload of the mitochondria and that's the end of it.

So this hypothesis is from '87. The same year Peter Hostman's lab produced results showing that if you have transient ischemia with recirculation, then you find that up goes calcium immediately after ischemia in the mitochondria. Then it normalizes, and then many hours afterwards the calcium content starts increasing. And Siden and Simpson have now shown that with certification techniques that the mitochondria show a secondary rise in calcium many, many hours after the transient ischemia.

So I think there is substance in this hypothesis. What's the problem? We assume that we have membrane dysfunction, and that membrane dysfunction could either lead to an increased glutamate release presynaptically or it can lead to an upregulation of, say, AMPA receptors post-synaptically. What we would get then would be sodium influx and depolarization, and then you'll get calcium influx by three, four, five, six different pathways.

If you look upstream with an N-type calcium channel blocker, SNX-111, the decrease is very rapid, even if you give the drug 16 to 18 hours after ischemia. If you give an AMPA receptor blocker, you also decrease the final cell damage, and you can give the drug up to 24 hours after ischemia in terms of the CA1 cells. So the assumption is that if you block upstreams, you reduce sodium influx, you reduce depolarization, and thereby calcium influx. And now it could possibly explain why there is no effect of an NMDA antagonist, because this acts downstream here and blocks only one of the many different pathways by which calcium can enter. So the trick would be to try to reduce glutamate release in the post-ischemic period or to block sodium channels, perhaps particularly a channel which is gated by the AMPA receptor.

This is unfortunately not all the story. This is from Carney and Floyd. In '88 and '89, they said let's try to prove that free radicals are formed in the recirculation period following ischemia. So they gave salicylate and looked at the hydroxylation of the benzoic acid molecule in the recovery period, or they gave a spin trap, PBN--and remember that compound now, please--and they looked at the EPR signal, and they showed without any doubt that if you

have 10 minutes of ischemia, then during the first 15 minutes of recirculation you do form free radicals. And in the gerbil they got results suggesting that the spin trap given to detect free radicals actually decreased also the brain damage.

Unfortunately, people like us and others could not confirm this in the rats, so there was some kind of confusion for some time. But I'll come back to that.

The important thing is that if you know this, then it is, of course, logical to give a free radical scavenger, and this is dimethylthiourea, which is given before--Shersta Fallmak's work from our lab--and there was a reduction in the cell damage in subiculum, in the CA1 sector, and not shown in the neocortex and in caudoputamen.

This is the hydroxide radical scavenger, and it reduces the cell damage after transient ischemia, so somehow free radicals must be involved in this also.

We then suggested that perhaps this is what happens. Calcium goes in during the ischemia, and then when you recirculate, calcium is pumped out, again, from the cell. But in the intervening period, you have triggered the production of free radicals, and they can now affect the proteins in the calcium channels or the calcium pumps so that you reset the balance and get your cycling of calcium across the membrane. But then by protein oxidation or lipid oxidation, then, of course, you can affect the activity of kinases and thereby affect ion conductances and gene expression and protein synthesis. Perhaps this is a way of not only getting mitochondria overload, but also of producing a DNA signal or a change in the gene expression.

Then we come back to apoptosis. This is from Martin, et al., and they showed that in vitro you turn on a suicide program and get apoptotic cell death if you withdraw growth factors from the medium. It has now been shown by many laboratories, both with global ischemia and with focal ischemia, that if you give cyclohexamide, which is a blocker of protein synthesis, you actually reduce the cell damage which results from it. So it's not inconceivable that we are dealing with two things here: one, with the overload of the mitochondria by calcium, but that may be orchestrated by something which reflects the expression of a gene, or activity of an endonuclease which would fragment to DNA.

With all that, I should like to say that when we are discussing long periods of ischemia and if you learn how to treat the major catastrophes resulting from that, then you may still have this program cell death, and, therefore, I think multi-therapy is washed up. We have to discuss it in the future.

The second type of ischemia--and now we are dealing with ischemia of intermediate duration, or ischemia where reflow may be set up. So we're approaching now the long ischemic periods that you are interested in, but I should like to stress that we can get the same bad results for the

same period of ischemia by adding hyperthermia as an insult or adding pre-ischemic hyperglycemia as a complication, and then we get no longer overly selective neuronal vulnerability. We get vascular damage. We get laminar necrosis, and we get infarction.

So I will take hyperglycemia as an example just to remind you, if you have complete ischemia and look at the amount of lactate accumulating in the tissue, you usually get values of somewhere between 12 or 16 on normal patients. But if you induce hypoglycemia before, you get less lactate. If you induce hyperglycemia, you get more lactate.

We know now that this gives much more damage than that does. If there is a trickle or flow to the tissue, then, of course, there is a continuous delivery of glucose, and you can get excessive amounts of lactate in fed animals or those infused with glucose, but not in fasted ones.

Now, this is a fascinating story. If you take rats, subject them--or cats or whatever it is, subject them to 10 minutes of ischemia, and if you add hypoglycemia to one group, you find--and this is specific gravity and this is time after 10 minutes of ischemia--that there is an initial edema in both normal and hypoglycemic animals. In the normoglycemia ones, then you get no secondary edema and only selective neuronal vulnerability. In the previously hypoglycemic ones, where pH fell more during ischemia, the initial edema is not very much more exaggerated. Now you get a secondary edema after 18 to 24 hours. You get seizures. The animals go into status epilepticus, and they die with grossly swollen veins and infarctions. So somehow this rise in plasma glucose concentration has done something.

Pin Yong Lee in our lab, a neurologist from China, titrated animals so that he had glucose concentrations of between 4.5 and up to 16 before ischemia, induced 10 minutes of ischemia and found that there is a very narrow threshold over which the damage becomes worse. This is 12 to 16 millimolar. He also found that in hypoglycemic subjects, now you recruited damage in areas which are not normally damaged, such as CA3 and single A cortex and substancion. So hypoglycemia does something which is really bad.

The question is now what's happening here, and I will introduce an old slide just to point out to those of you who are not familiar with it that if you make a mitochondria preparation and if you look at the oxygen consumption, you add substrate and ADP, you see an oxygen consumption; if you add an uncoupler, you see oxygen consumption. That would tell you something about the state 3 respiration, which is this, and the capacity of the mitochondria to generate electrodes.

Incidentally, this shows that if you reduce pH in your preparation, the state 3 respiration goes down and goes down. So low pH is not good for mitochondrial function, but this has nothing to do with the delayed damage after hypoglycemic ischemia. Of course, now pH is back to normal, and yet one sees what you can see here. That is, that if you

take 15 minutes of ischemia and look at the respiration of isolated mitochondria, there is a decrease in state 3 respiration. But if you recirculate after 15 minutes of ischemia, both normal and hypoglycemic subjects, they recovery mitochondria function, and this is with recirculation of 30 minutes.

This was published in 1986. Loterin was the first. He worked together with Amster. I think Amster was here during your last meeting.

We made one mistake here, and that was to carry recirculation through longer periods. But what we did--and this is Steve Venka's work--was to use complete ischemia which would correspond to normoglycemic animals, and you see that even after 30 minutes of ischemia, there is recovery of state 3 respiration. But not in animals with incomplete ischemia where you get more lactate. So low pH during ischemia prevents recovery of mitochondria metabolism after long periods of ischemia.

Now we have the results of Clark and collaborators from (?) , and I will tell you about them in a while. But I'll just ask the question: What could the reason be that we get more damage when acidosis is aggravated during this? I think the most likely possibility is that you aggravate free radical production. It's extremely easy to show in vitro that if you lower pH, you form more free radicals, and you can actually pursue more your Vitamin E content, it homogenates. If you just lower pH to 6.5, it homogenates. It can either be because you shift disequilibrium over to the right and you get more lipid soluble and more pro-oxidant radical than its anti. Or it could be because the hydrogen ions can displace iron from its bindings to transferring lipoproteins, and then you get catalyzed free radical reaction. This is a very likely possibility, but it is probably not the only possibility.

Dempsey from Kentucky published that with his group. They looked at gerbil ischemia and measured the messenger RNA for C-fos, one of the immediate early genes, and found that ischemia led to an upregulation or expression of C-fos messenger RNA. You have already heard that this occurs afterwards. They could suppress that by injecting glucose into the animals. So obviously the acidosis now also suppresses a program which the cell may require to survive after the transient ischemia. And Eastman and collaborators, I think from Vermont, have shown that, at least in some extracerebral tissues, low pH will activate an endonuclease, DNA-2, which is not sensitive to calcium or to magnesium, but which is sharply activated by the fall in pH in this general region. So we may have DNA changes, changes in gene expression, and we have free radical production. Actually, the free radicals may give rise to DNA changes.

The last part--and I think I would be all right on time so we can have a discussion, but now we are coming to long periods of ischemia, because I will be discussing focal ischemia where we have this focus and the penumbra tissues,

where cells are at risk. And we know that unless we pharmacologically protect these tissues, they will become part of the final infarct.

I think there's a general kind of agreement that cells in the perifocal penumbra area are threatened by two events. One would be regularly occurring ionic transients, and the second would be a gradual compromise of capillary circulation.

COLONEL BELLAMY: By long duration, do you mean an hour?

DR. SIESJO: Yes. I mean up to 2 to 3 hours.

DR. SAFAR: Focal.

DR. SIESJO: Focal, yes. That's right. Sorry, that was the first mistake. If you put in electrode in the perifocal penumbra zone of a focal ischemic lesion, many people like Gordon and others have recorded irregularly occurring ionic transience. So if you have a marginally perfused tissue, this ionic transience may give rise to cumulative damage and finally to infarction. At least this occurs in the rat. And some people now believe that the reason why NMDA antagonists and AMPA antagonist and unselected calcium channel blockers actually protect the penumbra tissues is that they prevent these irregular depolarizations from occurring. But that's a completely different story, and I will not bother you with it, but instead discuss why we know so much about reperfusion of the long periods of ischemia.

There has been a surge in interest in it simply because techniques have been developed for transient occlusion of the cerebral artery. This was described from Japan first, and Menesarba introduced the technique in our lab. You simply cannulate the carotid artery so that you can push a small filament up into the anterior cerebral artery; then you occlude the cerebral artery and you get ischemia with a massive fall of blood flow into focus and a slightly less massive fall in the penumbra zone.

If you now occlude for 30 minutes, 60 minutes, 90 minutes, 180 minutes, these are very early examples of what you get. But essentially with 30 minutes you'll get an infarction only in the caudoputamen focus. With 60 minutes, you occlude neocortical tissues. And with two hours, you get the maximum size of the infarct. Of course, it's the same as you see with permanent dilution.

So now we are dealing, in the rest of my discussion, with two hours of cerebral artery occlusion and recirculation after long periods. If you look at this, would you believe that it's possible to rescue that part of the caudoputamen? If you come in later than 30 minutes, would you believe that you could rescue any tissue after two hours? Three years ago, we didn't believe that. Now we know that is possible, that you can come in long periods after the recirculation and salvage tissue.

So let's at the PBN now, which worked in gerbil forebrain ischemia but not in rat forebrain ischemia, which is a free radical spin trap. In the experiments performed by Chi in our laboratory, cerebral artery occlusion of two hours was induced, and then we looked at early therapeutic interventions, with PBN given either before, one hour after, three hours after or six hours after the recirculation start. And the remarkable thing happened that if you look at the size of the infarct in untreated animals, these are the sizes of the infarcts you see. If PBN was given before, you clearly reduced the size of the final infarct. Some of them didn't have any infarcts as viewed 48 hours afterwards.

But what was even more remarkable was that if you gave PBN either one hour or three hours after the start of recirculation, you saw the same decrease in the size of the infarct. But after six hours, the effect was lost. So here you had a therapeutic window which was at least three hours.

Then we set up the hypothesis which we think is at least a working hypothesis. And I must say we were extremely stimulated by John Hallenbeck's early experiments with long periods of ischemia many, many years before all this started here. But we assumed that transient focal ischemia leads to infarction which can be ameliorated by PBN, even when given up to three hours. This suggests that recirculation sets in motion events which trigger the secondary damage. This is classical. I mean, it's reperfusion damage and nothing else.

But when we perused a bit the recent literature, then that suggested that the events which we are proposing here could either encompass plugging of microvessels with PMN leukocytes, and then we are leaning on Del Zoppo's work published in 1991 and later, or that we could have endothelial cell plus astrocyte swelling, something which has been proposed by Garcia.

Alternatively, we say that mitochondria could suffer secondary damage, and here we have just quoted Clark. Of course, I'm coming back to that. So this is the hypothesis.

What could we do to prove what is really going on? Then my friend, Fortagrova, who is an absolutely superb analytical chemist, we have worked together for more than 20 years, he came to--he introduced this to the lab so we could induce ischemia, freeze the tissue in situ, dissect out the perifocal penumbra cell and the neocortical focus for biochemical analysis.

Then the tissue was sampled either before ischemia, at the end of two hours of ischemia, and one, two, and four hours after recirculation.

It turned out that in the penumbra, control animals showed this decrease in ADP concentration after two hours of ischemia. After one hour of recirculation, there was some resynthesis of ADP, no more after two hours, and after four hours there was no indication of a secondary deterioration of energy state. In the focus, then naturally the ATP values

were even lower, as you see, very close to zero here. There was good recovery after one hour, but no further recovery after two hours, and signs of a secondary deterioration after four hours.

The remarkable thing was that PBN given one hour after the start of recirculation reversed it and led to resynthesis of ATP, both in the penumbra and in the focus. So a free radical spin trap given one hour after the start of recirculation could achieve this short-term improvement.

When we looked at the lactate, it was the same. There was a recovery of lactate and a secondary increase both in the penumbra and in the focus where we saw some excessive values after four hours of recirculation. But look at what PBN does. It really improves mitochondria recirculation, and that may explain why it reduces infarct size.

So the last slide is now getting our concept of two therapeutic windows. If you really go through the literature carefully and look at stroke, then it's clear that NMDA antagonists and AMPA antagonists are good. But the therapeutic window is very narrow, and you have to give the drugs essentially within 30 minutes after you induce the--

COLONEL BELLAMY: Sorry?

DR. SIESJO: You have to give it within 30 minutes after you clip the artery to have an effect. Therefore, we are suggesting that we have a first window, which is narrow, reflecting calcium influx, and we also suppose that this is triggering calcium-related events, that that will lead to production of lipid mediators, inflammatory cytokines, and free radicals.

Now, what you do with this calcium influx obviously is to start a grass fire. After one hour, when you have started a grass fire, you can no longer effect the whole thing by getting at the initiators. But you have to attack the grass fire itself. And that explains perhaps why calcium antagonists cannot stop these things.

The second window of opportunity we assume is large. It is three to six hours. Of course, there are certain drugs which can block the reaction solicited by the initial cascade, and we assume that these reactions either encompass the upregulation of adhesion molecules for PMN leukocytes, or that they prevent the secondary mitochondria failure. And as a result of that, we have been lucky enough to be able to set up an international task force working on this problem. We worked with Del Zoppo in San Diego and Forchinsky in Heidelberg on microvascular patency and microvascular events with the blocking of adhesion molecules. And we worked with John Clark on mitochondria protection.

Anyhow, this is where we are, and we think that the data, although they are not directly relevant to the problems you're discussing, they at least tell you that it's extremely important to come in with something that can block the secondary events after long periods of ischemia. The reason why PBN is active, we don't know. But PBN in contrast, for

example, the lazarens which are produced by the Upjohn Company, they penetrate the blood barrier without any problems, and you get a peak concentration in the tissue after 30 to 45 minutes following intraperitoneal. And then the compound is cleared from the tissue in 2 to 3 hours, so you have to give it by repeated injections. It may be that PBN is so efficacious because of its partitioning of the tissue, either in the mitochondria or at sites in the microvessels which are crucial for what is out there.

So this is where we are, and I can either tell you something very quickly--it might take me about three minutes--about fascinating therapeutical possibilities when it comes to mitochondria dysfunction, or we can wait until we have discussed this. But I'll stop here.

DR. HOCHACHKA: Just before you leave this particular point, I momentarily must have dozed off at 5:15 in the morning my time. I didn't write down what PBN was. It's a spin trap?

DR. SIESJO: Yes, it's a spin trap.

DR. HOCHACHKA: It's a spin trap molecule. What is it called again? Oh, it's in there. We've got it in your abstract. Sorry. I shouldn't have even asked.

COLONEL BELLAMY: Now, have you had any experience with other free radical scavengers of a similar role?

DR. SIESJO: Yes. DMTU we have used, but only if it--it would be interesting to look at DMTU, but DMTU is supposed to be a hydroxided radical scavenger. It penetrates into the tissue, but you have to give it in very large amounts, and the PBN obviously works in much lower concentrations.

COLONEL BELLAMY: Now, this is given with reperfusion or afterwards? What is the time sequence?

DR. SIESJO: Actually, we have results with good effect on the stroke sites when we give the drug three hours after we have started recirculation.

COLONEL BELLAMY: Oh, really.

DR. SIESJO: The effect one hour post-treatment is great.

COLONEL BELLAMY: We would have the option of infusing these substances at the same time that reperfusion or recirculation was begun.

DR. SIESJO: Yes. And I can see the advantage of using a drug like that. For example, in combination with something which would stop apoptosis, like cyclohexamide, and perhaps also giving something which would improve mito--sorry, microvascular.

DR. VIRMANI: My question to you is: Did you look at collateral circulation at all?

DR. SIESJO: No. We are doing that now, but the reason why we didn't do it immediately was that we have the same good effect with post-treatment.

DR. VIRMANI: What if you keep occlusion permanent and then you gave the treatment anyway? Maybe you can reduce the--infarct size.

DR. SIESJO: That's a good point. Let me see if I can get the slide. The last slide, which you didn't see, is kind of interesting, too. It needs to be confirmed. It was done from our lab from Don Phillips' group in Detroit. What they did was to use one of the techniques for permanently occluding the distal artery. Then they measured the infarct size after 48 hours. When they pre-treated or gave the drug 30 minutes after for five hours after, they had a highly significant decrease in the size of the infarct, although it is more or less a permanent occlusion. But they had some effect also with 12 hours post-treatment.

We still have to see if this is holding up for longer recovery periods. There's no question about that.

DR. VIRMANI: It would explain in a way that there is a low-flow state, so the cells don't die early and it takes a longer time to kill the cells.

DR. SIESJO: Exactly. Absolutely.

So let me just very quickly fill--are there any others of you who are not--yes?

DR. VERMA: I just have one more question. Aside from the NMDA receptor, magnesium is assumed to go out in a variety of calcium strips, including extra plasma membrane as well as intracellular calcium--

DR. SIESJO: Yes.

DR. VERMA: Just try to--

DR. SIESJO: I think we should do that. The reason why most people don't produce magnesium is that, like calcium, it crosses the membrane barrier--you more or less have to inject it into the tissue. But I think the group with Link and McIntosh, they have shown that after trauma, there is a decrease in the magnesium of the cell. You actually can ameliorate the damage by giving magnesium intravenous. So that is a good one--

DR. VIRMANI: Magnesium has also been shown to be effective in--reducing infarct size.

DR. SIESJO: That's interesting. For those of you who are experts in the field, this is just insulting. But just to remind you that we have the electron transport from the proximate carrier to cyto oxidase, and then we are supposed to form ADP at three steps, and substrates can either enter at this stage or they can enter a succinate at that stage. That's a very, very old slide, and to get a more accurate representation of what is today considered to be the important thing, that is, that we have essentially five complexes. Complex I is now the LDH, the hydrogenase. That

would take care of the electrons. That would be more forward, and Complex II will also deliver electrons. Complex III will carry them on, and Complex IV will then deliver the electrons to the cyto oxidase. Complex V is nowadays the ADP-producing step where the hydrogen ions kicked out of these places moves back passively and generates ADP.

Now, 60 minutes ischemia from John Clark's work, and the recirculation, the change in state 3 respiration during ischemia, recovery, and then after two hours of recovery one sees a secondary decrease in state 3. Never mind about that. So the mitochondria are affected, they recover, and then after two hours, they're getting sick again. And when John Clark looked at the complexes here, the enzymes, then he found that the Complex I and the Complex II to III, they are affected during ischemia, but then do recover, while there is a secondary small decrease after two hours. But after two hours, look at the Complex IV, which is cyto oxidase. So this is decreased now, and this is supposed to be an enzyme which is very sensitive to free radical damage. So it could be that the free radicals are really affecting mitochondria metabolism by hitting a special enzyme which is cyto oxidase.

There are many people interested in mitochondria metabolism, and some fascinating opportunities arise here. This is from Crompton's laboratory, and I have to correct that old figure because it should be ATP here and not ADP. But it shows the mitochondria membrane where hydrogen ions are pumped out, and then they leak back, producing ATP. Then there is a closed pore in the membrane, and when ATP is low, when calcium is high, PI is high, and where there is oxative stress, then this pore can be irreversibly opened, and then the hydrogen ions will not leak back, producing ATP, but they leak back passively, and then the mitochondria will generate heat that there is no way they can make ADP.

COLONEL BELLAMY: Like dinitrophenol?

DR. SIESJO: Exactly, exactly. But the interesting thing is that this pore can be closed by cyclosporin A. They found that out, and Richter in Zurich has another hypothesis. But in his hands, cyclosporin A can also lead to a correction of the mitochondria dysfunction that you see under conditions which resemble those in hypoxia/ischemia.

The last fascinating one is from Richter's laboratory with one of his review article. Richter got in contact with me after he saw the PNS article with had on PBN. He says we can have pro-oxidant, free radical-induced calcium release from mitochondria, calcium cycling by mitochondria, collapse of the electrochemical potential and ADP depletion, and that's the end of it. What you very often get then is apoptotic cell death.

Now it's been found that this death can be inhibited by cyclosporin A and by anti-oxidants. This step by--this is not interesting, but if we look at the collapse of the delta-mu and ADP depletion, it seems that over-expression of DCLA-2 will have an effect there. And so what

we have is a sequence of events where we will have on the one hand calcium influx, induction of apoptosis, and on the other hand a blockage of calcium influx and what you could get then is too--so now suddenly ischemia has become part of the discussion of how you reduce genes in the two states. So the thing has become extremely complicated, but at the same time extreme.

Thank you very much for your time.

COLONEL BELLAMY: Thank you very much. Very, very interesting.

COLONEL BELLAMY: We'll have time at the end of the session for further questions. I find most of your observations--all your observations are absolutely fascinating, but I think we're probably going to have to delay further questions because of time constraints, to this afternoon. You have to leave early, don't you?

DR. SIESJO: No. Nine o'clock tonight.

COLONEL BELLAMY: Very good. Dr. Hallenbeck will speak on hibernation.

MR. GRAY: I have one additional administrative thing to pass on. For those of us who are sitting in the bleacher seats and we have a question, we've got to really make sure we sound off--when Dr. Verma asked his question, Chris had a little bit of trouble picking it up. So if we ask questions from the bleachers, we've got to project so that we can be picked up.

DR. HALLENBECK: Maybe I'll just go ahead. Ron Bellamy came out to the NIH and talked to me about this meeting, and he was interested in work that we published on cerebral blood flow in hibernating animals and some thoughts that we had about how hibernation could relate to treatment of stroke. That's one of the areas that we're interested in. And I thought perhaps that laying out our concepts about this could be heuristic for you as you're thinking about how to approach this problem of suspending animation, basically, in people with these battlefield wounds. I think to be juxtaposed to what Bo has talked about will be helpful for this.

You will recall that in describing a focal lesion, Bo mentioned that there is a central core. If you have prolonged dense ischemia, you get a central core of necrosis where the flow is so low that the thought is that the tissue dies very rapidly, and it's regarded as essentially irretrievable. Now, that is, of course, the area that you are trying to retrieve. So in this sense, your goals are somewhat different than the goals of those who are trying to treat acute stroke. But we're conceding this core at this time, and then collateral circulation will bring in flows which do not support function but do not lead to rapid loss of ion gradients, so that these cells are viewed as in a metastable state. And as Bo went through very carefully and

with documentation by evidence from his own laboratory, the death in this region unfolds, at least in rats, over a period of hours rather than minutes, and so this would give time for intervention, and in people this seems to be even a bit longer.

So the question is, you know, what can you do to stop progressive damage in this zone, and even though it does differ somewhat from your goals, I think that the principles that are effective here are things that certainly ought to be tried in circumstances that you are interested in. So it's not utterly irrelevant.

But another thing that I think if you were listening carefully to Bo that might have struck you is that if you just take what is known about progressive injury in this region now, it's not a simple thing. You can't point to a single mediator or factor or something that's causing the problem. It's extremely multifactorial, and this would be a non-exhaustive list of things, many of them mentioned by Bo, for which there is some evidence for their participation in progressive injury in the ischemic penumbra zone in focal ischemia. You can read them as well as I can. In the early 1980s, Bo's article on The Mechanisms of Cell Death, A Speculative Synthesis, just turned the field into calcium, and there has been tremendous interest in that since. Excitotoxins have taken over since that time. So the newer things involve inflammatory mediators, altered gene expression, some things being actually beneficial, others leading to things like apoptosis. But as you can see, there are an enormous number of things, and there are things that are, of course, missing from this. A lot of interest in nitric oxide and then there will be mediators that have not been even described at this point.

In general, if you've been in the field for a while, you see a pattern that emerges. People will become enamored of one or another of these mediators for their own reasons, and they'll champion this mediator as the cause of the progressive damage. They'll concede that a lot of other things are going on, but if we just manipulate this particular mediator, we'll account for a lot of the problems and maybe we'll have a pronounced effect.

The way the sequence usually unfolds is that there will be somebody who is pretty convinced that this is true, and they'll use an animal model which is sensitive to small effects, and they'll get outstanding results, publish it, and then perhaps other people in the field will sort of seize on this, but they might not be quite so invested in the hypothesis. So in their hands, the outcome is maybe not so spectacular, and the range of outcomes widens. Then if it survives this kind of testing in multiple labs and gets to people testing, it all washes out.

This has been a pattern that we've seen, and maybe some of you interested in other fields have seen the same sort of thing when you're dealing with this problem of ischemia and hypoxic damage and so forth.

Now, the problem could easily be that we just haven't found the dominant mediator or controlling factor. That's certainly possible. But a possibility that cannot be at this point categorically excluded is that there is no dominant mediator, that, in fact, these things, which in aggregate are tremendously potent, are all acting as a constellation of minor causes. So that if you go in and block any one of them, you don't have the profound effect that you want.

I would say, since the idea here is to generate concepts and discussion and so on, I am very aware that to think about this is a very vulnerable hypothesis because all that has to happen is for one of the promising initiatives that are going on now to succeed, and then this is absolutely wrong. But until that happens, this remains a possibility.

The reason I think to have some people considering the possibility is that if it were to be true, if it's the true nature of the problem, then almost all of the research that is currently being conducted along these lines is doomed to fail. So there would be a reason, even if it's a long short, for some people to be hedging against that possibility. That's sort of why we're doing this.

I think those are sort of the main points in the introduction. At any rate--I may have to just run that manually, I guess.

COLONEL BELLAMY: I think so, unfortunately.

DR. HALLENBECK: If you decide that there is a possibility that the true nature of the problem could be that it's caused by a constellation of minor causes, then the next step gets very difficult because we're absolutely lousy at trying to solve problems that are due to constellations of minor causes. Our approach is always the same. We try to find something that accounts for most of the variability.

So there may be many ways to do this that we certainly haven't thought of, but one way we thought about was that if you look at nature as having eons of time for trial and error compared to the lifetime of one investigator, if somehow nature has solved the problem or seemed to have solved the problem, perhaps we could learn from the way it has done it.

To us, hibernation was attractive in that regard because there are membrane adjustments, metabolic adjustments, and circulatory adjustments and so forth that enable these animals to live under conditions of trickle blood flow through the brain for very prolonged periods of time with no apparent damage. And so if we can understand how that is regulated, it is conceivable that it could be heuristic, that could guide us.

For our work, what we have generated as hypotheses is that this state of hibernation does involve--it's not as I think one of the investigators, Larry Wong, I think has said, that it's not a reversion sort of to primitive quakal thermia. It's a controlled state, has endogenous mediators,

and some of these mediators could perhaps produce tolerance in homeothermic species.

Next slide? So we thought what we'd do is just try to characterize the state, see if we could find some of these mediators, and then see if they were useful in models of focal ischemia, and we're proceeding along those lines, but I can tell you already that we have not certainly cracked this. But we're certainly working on it.

Next slide? I'll just go through some of the physiology. There have been a lot of people who have worked on this and have shown these things, and then just go through a couple of--some of the work on trying to characterize the media.

All right. So the animal that we're looking at is this thirteen-lined ground squirrel. I grew up in Minnesota, and these would be called gophers in my home state. In fact, the football team is the Golden Gophers.

If you take these animals and put them into a--well, you can operate on them initially and put in these multichannel transmitted which will record ECG, EEG. We initially were trying to get CSF, but it's harder to get CSF out of a ground squirrel than it is a rat, so we didn't do real well with that. We could put in catheters, and you could take advantage of the fact that when they hibernate, they tend to curl this way. So if you just tunnel under the skin and bring the catheters out through the back, you can have access to them without disturbing the animal.

Then you have a chamber, which is a hibernaculum that we could go into, an environmental chamber. It was kept dark except when we were doing procedures we had a little red light, a photographic light. But, otherwise, it was dark, kept at five degrees, and so on, and then we could have 12 to 15 of these telemetry devices that would be sending signals to a high band multichannel receiver, feed it through a matrix to a computerized system that would record EEG, ECG, and these various things.

Just like rats, the ground squirrels have normally very rapid heart rates. This is in the range of 360, 370, for those of you that lack sort of a Rainman-like capacity for analysis of rapid counting of things, but the startling thing is that when these animals go into hibernation, they have enormous changes so that the heart rates can fall down to 1 percent of what they were normally. The EEG will absolutely flatten.

The rate that they go into it is kind of interesting. Within something of the neighborhood of 30 minutes to 2 hours, they can make the transition, and we were struck that the heart rate was dropping faster than the temperature, suggesting some regulation going in. And people have noticed this also with the drop in oxygen consumption.

Then coming out, it's the same way. It's very rapid. But the heart rate change precedes the change in temperature.

DR. LEWIS: Where did you measure body temperature?

DR. HALLENBECK: The temperature sensor was under the skin on the back.

DR. LEWIS: Did you measure brain temperature?

DR. HALLENBECK: We didn't have an electrode in the brain, a temperature probe in the brain.

COLONEL BELLAMY: What was the core] temperature? Or how does the ambient temperature relate to the core temperature of the animal?

DR. HALLENBECK: What happens is the animal's core temperature, which usually comes to about 1 to 2 degrees above ambient, and it does control.

DR. HOCHACHKA: But these patterns for core temperature are exactly the same as you're reporting here.

DR. HALLENBECK: Right.

DR. HOCHACHKA: The exact value may be slightly different, but the pattern is the same. It always tracks behind the metabolism or the heart rate or whatever, so that the whole thing is body temperature is, in effect, a change in metabolism.

COLONEL BELLAMY: The obvious question is: What happens if the temperature of the room did not change?

DR. HALLENBECK: The animals--first of all, this kind of process doesn't only happen in the cold. There is estimation and things of that sort. Now, animals don't generate cold, obviously. The best thing to do--

COLONEL BELLAMY: Unfortunately.

DR. HALLENBECK: They can only come down near ambient, but it would usually be that they would come down close to whatever ambient is and stay there.

DR. LEWIS: The reason I asked about this, is that core temperature and brain temperature might not necessarily follow each other.

DR. HALLENBECK: Yes, that's true. It's particularly true when you're causing ischemia and so forth. It's very true. In an animal that's intact, probably these temperatures are very close to core temperatures. The animal comes down, as I say, to within a degree or two of ambient. I don't know if maybe--you know, about the distribution of temperatures in the body. I don't think there's much non-uniformity, though. I mean, there isn't much for them to operate--you know, everything is within a degree or two of ambient.

COLONEL BELLAMY: The liver is certainly warmer than the rest of the human body, at least.

DR. HALLENBECK: The what?

COLONEL BELLAMY: The liver is about 101 degrees Fahrenheit, usually, and the rest of the body is more like 98--the core, that is. The skin can be 70.

DR. LEWIS: The brain is cooler.

COLONEL BELLAMY: There is some suggestion that there is a mechanism for cooling the brain, isn't there?

DR. LEWIS: As the temperature goes up, the brain temperature does not go up as fast. This was the question. If the core temperature goes down, does the brain temperature go down as fast or not? I think it would be interesting to know.

DR. HALLENBECK: We haven't asked that question.

MAJOR BRUTTIG: Another thing, though, in trying to get back at Colonel Bellamy's question: What happens if you kept the chamber cool, do you still get these changes? One of the things probably impacting this is that you are using a hibernator, and hibernation is a survival technique for cold, darkness, lack of food, et cetera. So it's one of the triggers which causes this event.

DR. HALLENBECK: Right. True.

MAJOR BRUTTIG: And so if you maintain cold in that chamber, you're liable not to get the recovery, the release from hibernation.

DR. HALLENBECK: For periods usually of a few days up to a couple of weeks, there would be a bout. These things occur in bouts, and even though the environmental conditions are maintained, they will then come out, and there is some interest in what--why do they periodically have to emerge and then go back? What is happening? That's not really known.

DR. CHAMPION: So how did you trigger this? And it's purely environmental.

DR. HALLENBECK: Yes. These animals will--you can make them hibernate primarily during a season, there are circannual rhythms that they have, and they are really trying to hibernate starting in what would be the cold season normally in the area that they come from. But if you put them in even in the summer, they will--it takes longer and it's less predictable and so forth, but some of them will hibernate. But most of our work is conducted during the hibernation season, and during that period it just takes a couple of days to put them in and then they'll go out normally. There is some individual inter-animal variability.

One of the things that fascinated us--and I might just mention a dimension of this--most of the interest in a state like hibernation has been in the fact that, say, unlike hypothermia alone in which you have, say, differential effects of cold on interdependent processes so that you come out of homeostatic balance, everything is maintained in hibernation. The energy supply is matched to the energy demand and so forth, and homeostasis is pretty well maintained. But they do do some things that you could

interpret as sort of hedging against damage, and this would be along the lines of--you know, if there's damage to a tissue, you could imagine that flares go up, there is a kind of--the tissue starts to do things that are sort of self-destructive. It's perhaps best seen and most eloquently stated, I would say, in Louis Thomas' account of endotoxin. Here he would say if you just take the endotoxin molecule, you know, it's not very impressive as a direct toxin if you compare it with sulfuric acid or some sort of other thing. But it's a piece of misleading news. It's a false alarm, and the body breaks everything out of the armory and starts self-destructing. Maybe these hibernators are doing things that blunt their tendency to do that.

We're interested in the stroke field in, for example, neutrophils and platelets, monocytes and so forth, and although it does not really change particularly its circulating red cell mass, it makes huge drops in the platelet count, neutrophils, lymphocytes, and drops its monocytes down to a very low number. And it does this--if you remember the curves of the entrance and emergence from hibernation, these changes are made very quickly and with similar time curves.

So we're kind of interested in how it's doing this. Most of the binding of these sorts of cells, they're receptor-mediated, so we're interested in what's going on.

Another area, with the help of Lou Sokolov's group, the Laboratory of Cerebral Metabolism in NIMH, we did some blood flow studies, anti-kirine studies, and the squirrels have a little lower perfusion of their brains when they're active than rats. It would be closer to primates, 60, 70 ml/100g/min.

But when they hibernate, there's an enormous drop. There are regions that are hardly being perfused at all over a two-minute period, and it's about 10 percent of the flow during normal. These rates would lead to rapid brain cell death in an animal that wasn't hibernating.

I'll just show the difference here--

DR. HOCHACHKA: Can I just sneak in one quick question? Has that kind of blood flow experiment ever been done with estivating animals, normothermic?

DR. HALLENBECK: No, and we wanted to try to do that. Some of the hibernators--during the hibernation season, if you just keep them at 22 degrees in a normal room, they'll do these test drops, they are called, where they become somnolent and so forth. But the difficulty you have is the Heisenberg principle. When they're not really deep, any of the slightest kind of manipulation brings them out of it. So we found it hard to do.

We looked at glucose metabolism. What we hoped really was to use this elegant technique to find maybe regions, if we could, that stayed active during hibernation and might be controlling the area, you know, controlling the state.

But we got very hot-looking autoradiograms that were uniform, and what it turned out was that it's all precursor. This deoxyglucose flooding the brain, almost all of it is unmetabolized. We needed to have Jerry Deeno's techniques for direct measurement of deoxyglucose to find that the--and then do the processing to find that there's a tremendous drop in the cerebral--

DR. HALLENBECK: These are awake ground squirrels. One of them is just in its cage at room temperature. The thought is that you could have cold-adapted awake animals and what does the cold environment do to it. So it isn't a great deal it does to it, but certainly when they hibernate, it's a tremendous change.

DR. SIESJO: John, could they possibly use other substrates than glucose? Is there any evidence that ketone body concentrations would rise during hibernation?

DR. HALLENBECK: The beta hydroxybutarate goes up some, and gamma hydroxybutarate I think goes up some. We haven't looked at that. That's an interesting question.

Then we really tapped on Lou Sokolov's lab a lot. Caroline Smith in that group has been working on techniques with LUCY and C14 to look at the rate of cerebral protein synthesis. In awake squirrels, you could use her technique pretty well, and this level of protein synthesis would be similar for a rat. This is an awake ground squirrel.

In local protein synthesis, this ceases to be useful, and this is interesting. Bo talked about cyclohexamide and talked about suicide programs and so forth. Whether this has any survival value or not, I don't know. But on the other hand, Peter Hostman would say that one of the earliest changes in ischemia is the loss of protein synthesis and that's bad. I mean a reduction, but never like this.

If you scrape off the protein and count it separate, don't try to deal with it autoradiographically, there is about a thousand-fold reduction in the amount of protein synthesis. There still is some very slightly protein synthesis.

The point is that these animals don't just survive this kind of thing for a few hours. They'll go weeks at a time, and then when they come out, if you look at their brains--and we would be accustomed to thinking of the CA1 region of the hippocampus as being the most sensitive area to ischemia--there is no difference in the way the CA1 reading looks after hibernation than before hibernation, obviously in separate animals.

So our approach has been to try to understand how this phenomenal state is regulated, and we have looked--I'll just give you some examples. We were interested in, for example, the effects of--is there anything in hibernating plasma that would affect cultured microvessel endothelial

cells and alter their expression of known adhesion receptors? So we have cultures of rat microvessel endothelial cells, and we looked, for example, at some known agonists and compared their ability to upregulate the expression of ICAM-1 with cells.

Now, cells in culture are a little stimulated anyway, so they have some ICAM-1. But there's a pretty good upregulation with LPS and TNF-alpha. Those are two commonly used agonists.

But if you just take hibernating plasma and incubate it with the endothelium, it causes a big increase in ICAM-1. Even non-hibernating plasma had some effect, though if you dilute it out, its effect disappears, and it seems to be more in the hibernating plasma. And this carries over to monocyte binding. It's the same sort of thing. You have some monocyte binding to cerebromicrovessel endothelial cells that are not stimulated. You can increase it with TNF-alpha. Then the most impressive stimulation is with hibernating plasma, a little less with non-hibernating winter, and still less with non-hibernating summer.

I guess our interest in trying to--we're trying to fractionate plasma and see what's involved in this. If we can understand this process, it seems that what they do is park their leukocytes. Normally, if a leukocyte binds to endothelium, it's more than just tethering. When the ligan on a leukocyte finds its counter-receptor on the endothelium, there is actual stimulation and intracellular signaling that occurs, and there's a process of activation that occurs in the cells. And this doesn't seem to happen in the hibernator. So we'd like to know how that works.

DR. BAZAN: You have fewer PMNs, do you? You show us--

DR. HALLENBECK: Yes, yes.

DR. BAZAN: Far fewer.

DR. HALLENBECK: Right.

DR. BAZAN: And yet ICAM-1 is upregulated.

DR. HALLENBECK: Right. So the point would be that these cells are marginating to, you know, all around in the circulation, so they are taken out of the circulation. They're not available to be recruited into an area that becomes injured. And they don't seem to become activated and undergo transendothelial migration because when the animal comes out, they pour back into the circulation and--

DR. BAZAN: You say it's selective expression of ICAM-1. Is there selectivity insofar as different organs?

DR. HALLENBECK: We have not looked at that. The other step that I think is critical to this is to--there are indices of activation of leukocytes tissue factor expression, for example, on monocytes, maybe generation of super oxide, things of this sort, that can be measured. And so if you characterize, say, when leukocytes bind when they're

stimulated by TNF versus when leukocytes bind when they're stimulated by the hibernating plasma, what we hope to see is that the activation doesn't take place here, that it's just bound in part. We haven't done that yet.

The other area--and Peter Hochachka has discussed this, and a good deal of Bo's talk dealt with the idea that calcium entry is very important in ischemia. If you have to cut down on the metabolic demand, one of the things you'd like to do is change the conductivity of membranes so that you don't waste a lot of energy pumping. So we wondered what synaptosomes from hibernators would be like versus non-hibernators. What we found was, if we isolated these synaptosomes--is everybody familiar--some of you are not neural scientists. Are you familiar with synaptosomes? I should mention it if you're not. Okay.

What you can do in the brain is, if you do a kind of gentle homogenization, you can shear the processes of neurons, and you break up cells and so forth. And if you do various centrifugations, you can get a fraction that is very rich in processes of neurons, and these processes in aqueous solution will reseal so that what you'll have is the kind of number boost slide where he had a presynaptic portion of an axon, and then he had perhaps a dendrite, which is the post-synaptic. So he had kind of a synapse. Those things can shear off, reseal, so that you have a unit that has a presynaptic portion and a post-synaptic portion. And you can study it for entry of cations into it. You can study it for release of transmitters and so forth. It's a fairly commonly used preparation.

So we wondered whether we could isolate these, and, of course, one of the problems is it takes a while to isolate synaptosomes so that some of the characteristics of these processes in the body may be lost during the process of isolation. But we wondered if there would be enough retained that we could see any differences.

When we incubate these in medium for 5 minutes with radioactive calcium, calcium-45, and look at the rate of accumulation into these processes, there is--these are carried out at 37 degrees, so this isn't a difference in temperature or anything. The non-hibernating synaptosomes take up calcium faster than the hibernating, so there is less conductivity for calcium and synaptosomes of hibernating animals.

COLONEL BELLAMY: Could you give us an idea of what the scale is. We're told there is a one nanogram, I guess--I can't read it without my glasses.

DR. HALLENBECK: Nanomoles per milligram.

COLONEL BELLAMY: How does that compare to the normal intra-synaptosome calcium level? Is that 1 percent or is it 100 times? Do you have any idea.

DR. HALLENBECK: I can't tell you at this point. I certainly will look that up.

DR. SIESJO: You know, this is 45 calcium, sorry, I mean, do you have the count of calcium?

DR. HALLENBECK: No, no.

COLONEL BELLAMY: Trace amount--

DR. BAZAN: Trace amount.

DR. HALLENBECK: This is--we're not trying to characterize the total amount. We're looking at movement, differences in movement across.

COLONEL BELLAMY: So all we know is that there is an increase movement in the non-hibernating--

DR. HALLENBECK: Right, right. Yes, so I don't--this couldn't be thought of as characterizing the amount in the cells. There's about a 10,000-fold difference between inside and outside.

Okay. Then we if--

DR. SIESJO: With the synaptosomes, John?

DR. HALLENBECK: No. Normally in the neurons, not in the synaptosomes. I think that would be--

DR. SIESJO: No, but you are looking at 45 calcium, which means that what is interesting in terms of exchange between the outside and the inside would be total calcium content.

DR. HALLENBECK: That's right.

DR. SIESJO: But this would never give you any indication about the free, cytosolic one, but it would tell you how much calcium is translocated in, and then this will be diluted with the whole content, whether it is bound or sequestered.

DR. HALLENBECK: That's right.

So what you were looking at was the rate at which calcium entered, calcium-45, entered synaptosomes under non-depolarized conditions. Now, if you depolarize with 50 millimolar potassium chloride in the medium, again, the rate of depolarized calcium entry is less than in the hibernating animals, indicating that the voltage- sensitive calcium channels are in some way inhibited.

We tried to characterize which ones these were. Here is the inhibition that's seen in calcium entry in 5 minutes after depolarization. Cadmium chloride is something that just generally interferes with voltage-sensitive calcium channels, and that inhibits it. So you could inhibit both the hibernator, which is here, and the non-hibernating calcium channels.

Nifedipine, which is an L-type channel blocker, didn't have much effect. This is a clonotoxin from Clonus Geographus, and if this were an N-type channel that we were dealing with, this would have blocked it, and it didn't.

We got some inhibition with another clonotoxin that blocks N, P, and Q channels, and if we go one further with a funnel web spider toxin--this is agatoxin--in a low dose, this will block P channels, and it didn't. So the suggestion is that the ground squirrel synaptosomes are--the voltage-sensitive channels that it's dealing with are the Q channels, and it in some way inhibits those. Obviously there's more to do here, but I think of this as an example of the potential benefit of this kind of thing. As Bo showed so clearly, if you're sitting in the armchair trying to reason with diagrams and so forth, where you should intervene, and you see that there are all kinds of calcium channels and things, it's heuristic to see how a system that works works; you know, what it focuses on. Perhaps Q channels are important to think about. They've just been described recently, and not a lot of work has been done with them. But maybe this animal is telling us that we should pay some attention to it.

COLONEL BELLAMY: At least one place where, let's say, the hibernation trigger works is on the ion conductivity with a particular calcium channel? Did I hear you say that?

DR. HALLENBECK: Well, no. You're thinking of Okin's work, right?

COLONEL BELLAMY: No, I'm not thinking of anybody. I'm just thinking about what was said right here. The obvious question is, if there is such a thing as a hibernation trigger, whatever that might be, where does it work? It must be rather specific, I would think.

DR. HALLENBECK: Well, I'm not so sure that would--I mean, the thing--you know, the way it seems to me is that it might not be specific. It might have features that, say, cytokines have. It might be very cleotropic, because part of the problem here is that you have to do so many things at once if you're trying to stop progressive injury if you link this. Many changes have to occur at one time. An analogy I've used before is the problem is like an inner-city riot. Somebody's robbing a store, somebody's burning buildings, somebody's raping somebody and so forth. You come in and say--the big problem is the fires, you know, so you come in here with a fireman, but he's getting sniped at, and now these other things are going on. You need somebody that comes in and just says relax, be happy, you know, calms everything down together. Somehow I think this is kind of doing this sort of thing.

I have just a couple more slides. Basically, if you use the amiga clonotoxin that's M7C, this shows a concentration curve of inhibition which you'd expect with a Q channel. And only at very high dose agatoxin do you get inhibition of the channel in hibernators. So it seems like it's a Q channel.

The other kinds of things that we're doing to investigate this are--it does seem to me that the tools area available more than ever now to actually find out what regulates something like hibernation. We're looking at the short-term signaling like phosphorylations of proteins.

We're trying to do some molecular things, differential hybridization of subtraction libraries and differential display and so forth. Those are in progress. But I do think that if hibernation could be understood, it would have potentially a lot of biological applications.

DR. SIESJO: John, is there any information on post-synaptic calcium influx in the membranes from hibernators?

DR. HALLENBECK: We have no information on that. The things that I've seen don't really discriminate very well. Not that I know of, I would say.

DR. BAZAN: Any information, John, about the release of leukolate?

DR. HALLENBECK: Again, we haven't done that, and I have not--for example, by brain dialysis or something.

DR. BAZAN: When--or just give the primary cultures of the hippocampus neurons as we've done it, and look at the mechanisms, because that might give you an indication of endothelial arrows that could be critical in the early phases, and you might be able to assay by that approach potentially endogenous--I am thinking about phospholate, say two or three a year. Have you tried to see phospholate slow down in the brain?

DR. HALLENBECK: We haven't, and we would have to gear up--I mean, those assays are not going. Maybe--

DR. BAZAN: One quick approach would be to mention the products.

DR. SIESJO: The effect of calcium channel blockers--I mean the Q-type channel blockers--they are very interesting, but I wonder if this is really the problem that you are interested in. Because my hunch is that if you have really seriously ill patients who bleed out on the battlefield, then all the calcium that is going to get into the cells has already gotten into the cells at the time of--

COLONEL BELLAMY: Within a few minutes.

DR. SIESJO: Yes. So actually what you're looking at would be the effects of the calcium influx and the rise in calcium--

COLONEL BELLAMY: Which is what you addressed.

DR. SIESJO: Which is the cascade. And so this is just a comment. But I have one other thing which I had hoped I could bring up, because this was my reaction when I saw your slide on the white cell counts. David, we have discussed this before, not you and I, but in this field. When I mentioned Peter Hostman's 60 minutes experiment where he was able to prevent infarction, probably then to prevent non-reflow in a lot of the capillaries, what he actually did before he released the clamps, he raised the pressure to over 200 millimeter mercury in the capillaries. He lowered the

viscosity of the plasma, corrected the pH so he would have no coagulation problems or minimized the coagulation problems. My suggestion to Peter was that these procedures actually prevent, by instituting this procedure, would be adherence of PMNs to endothelial cells and plugging and preferential perfusion of microvessels. So one wonders how can a squirrel get rid of blood flow which is 10 percent of control and restart the whole thing without having microvessel problems. A beautiful way of doing that would be to sequester the PMNs and the other cells which could plug the microvessels when you restart circulation.

DR. SAFAR: Hossmann's model includes elevating the head during bilateral carotid and vertebral artery clamping. So there is drainage of much of the blood in the brain.

This is not normo-volemic arrest; it is exsanguination arrest. Could this be better tolerated?

DR. SIESJO: Could be, yes. But my point is that you're probably flushing the results and preventing any problems which would arise when you have a sluggish reflow and where you have an inflammatory reaction in the microvessels.

COLONEL BELLAMY: You would assume, though, with the high pressure you would actually have a more detrimental effect. That is, with the high perfusion pressure you would cause- because of membrane damage, you would have massive flooding of the extravascular space in the brain. In cardiac reperfusion, the high pressure of reperfusion is very detrimental.

DR. VIRMANI: People have done lower leukocyte counts and looked at reperfusion in the myocardium. It does decrease the amount of reperfusion, but it doesn't prevent it totally. So, yes, you can have beneficial effects from that, and people have done a lot of work with ICAM and they have done antibodies to ICAM and have shown that they can again, reduce the--infarct size but does not prevent it.

DR. SIESJO: The points I made previously was that perhaps they're dealing with two types of damage, and what you can prevent is the kind of mass destruction with necrotic patches in the tissue by preventing the no reflow while you can't do anything about it in terms of neuronal damage or selective vulnerability.

DR. VIRMANI: How do the leukocytes come back?

DR. SIESJO: About the same. You remember the entrance into hibernation and the emergence?

DR. VIRMANI: Yes. Same way?

DR. SIESJO: Now, we weren't able to get the blood immediately out but within, say, 30 minutes or an hour after coming out of hibernation, they're back.

DR. HOCHACHKA: So it's clearly a storage, isn't it?

DR. HALLENBECK: Yes, yes. It wouldn't be colony stimulating factors and that sort of--no. It's--

COLONEL BELLAMY: Are they sequestered in the spleen, for instance, or in--

DR. HALLENBECK: The amounts--we have done nothing to look at that. But some people have looked, and I think the liver and the spleen have quite a bit. But it wouldn't surprise me if it's kind of all over.

DR. LEWIS: Could be in the blood vessels also.

DR. HALLENBECK: Oh, yes. It would be marginated along--

COLONEL BELLAMY: They don't go through the wall, though.

DR. HALLENBECK: Right.

COLONEL BELLAMY: They don't cause a problem--

DR. HALLENBECK: See, that's the big thing. Normally, if you have ICAM expressed and there's an ICAM interaction, there's activation of the leukocyte, and it does undergo transendothelial migration. The fascinating thing to me is that maybe a mechanism has been worked out here for parking the leukocyte for a while and not activating--

MR. FALK: Transendothelial migration depends on other adhesion molecules as well as [inaudible comment].

DR. HALLENBECK: Right. But if you take the current ideas of selecting, ICAM and integrants, and then a certain amount of programming that takes place and so forth, and then other molecules. Somehow this isn't happening, just the initial bindings occur.

DR. LEWIS: This is happening is the small region of the micro-circulation. You could park them in the large vessels.

DR. HALLENBECK: Yes.

DR. LEWIS: Could I ask another question? I'm noted for asking questions that nobody can answer.

DR. HALLENBECK: I'm noted for not giving answers.

DR. LEWIS: There are animals that hibernate and animals that don't hibernate. You're telling us how they go about hibernating. Why do they hibernate? Why do some do and some don't?

DR. HALLENBECK: Well, you know, this gets into sort of theliological argument. I think as it is written about, people have calculated sort of the energy savings of these small animals by hibernating, and it's considerable. So it's a strategy to get--when food resources are low and it's cold, it's a way of getting through that period. And it's--

DR. LEWIS: But you were making the point which I think is very important for this group; namely, that there

are animals that have solved the problems we're looking at, and the question is then, the animals that don't hibernate, how do they survive this situation?

DR. HALLENBECK: Well, let me take the first part of your statement, if I could, because I don't know that I've shown that this animal has solved the problem you're looking at--

DR. LEWIS: Well, hopefully so.

DR. HALLENBECK: I think that some of the strategies could be applicable, potentially, but if you--I mean, the PO2's, for example, in the animals are--even though they're down at 1 to 8 or 10 percent of the heart rate that is normal, in deep shock, their blood pressures are maybe 30 millimeters of mercury, they're breathing very occasionally so that the oxygen delivery is very low, they've set their oxygen requirements at such a low level that they're not really stressed, at least as far as we know. Now, that could be investigated further to see whether there are--you know, if you follow it along, there may be times when they get stressed.

I mean, there are some differences between this and between absolute cardiac arrest.

COLONEL BELLAMY: Could I ask that we hold our discussion until the afternoon session? We can take our break now. I have a question. Dr. Safar has a question. Dr. Hochachka has a question. We all have questions. About 3 o'clock, we're going to bombard you with questions. Let's take a 20-minute break.

COLONEL BELLAMY: I think we're ready to begin the second part of the morning session. The first speaker will be Dr. Safar from Pittsburgh, and his colleague, Dr. Tisherman, will follow during the same hour period.

Dr. Safar?

DR. SAFAR: I want to start thanking Bo Siesjo for his ongoing inspirations on mechanisms, and I want to thank Dr. Hallenbeck for having been the mentor of my successor as director of our little multidisciplinary resuscitation research center.

By way of introduction of myself, I am a past anesthesiologist who had a little experience in resuscitating people outside the hospital and much experience resuscitating people inside hospitals. My research orientation has been that of an old-fashioned plumber (pathophysiologist). I am neither an electrician (cardiologist) nor a cook (biochemist). I am looking at the biochemists who are working at the molecular level with awe. But I am trying to learn their language, at least.

I want to share something with you concerning our programs. They are not only related to cardiac arrest—but also to trauma. There is a linkage, of course, in the topic

of concern to the military, namely in exsanguination cardiac arrest.

Since last summer, when my colleague, Dr. Kochanek, became director of our center, brain trauma has begun to assume a considerable portion of the center's efforts. My suspended animation studies began with dog research in 1988, under the then research fellow Dr. Tisherman as team leader, from him you will hear a little later.

We now have, mostly through the molecular orientation of Pat Kochanek, rather impressive collaborators, like Drs. Graham and Simon on apoptosis, Dr. DeKoskey on brain regeneration, and Dr. Kagen on free radical scavenger reactions. Much of what is going on at the cellular/molecular level in the brain trauma area will now spill over into the cardiac arrest area, into our studies with rats and dogs.

We must differentiate between protection (pretreatment), preservation (intra-insult treatment), our resuscitation (treatment to reverse the insult and support recovery). Suspended animation is the sequence of all three.

It is important to know how man dies slowly, not with ventricular fibrillation, but slower, over 10 minutes, as it may happen hour exsanguination in the field.

A year ago, we conducted a discussion session in Pittsburgh which was quite exciting. It will be published in the Critical Care Medicine journal in February 1996.

We are here today to determine the feasibility and advisability of coordinated multicenter, multilevel research on suspended animation.

To make the normothermic traumatized exsanguinating person maintain viability of his organism, including his entire brain (not just the hippocampus) during controlled cardiac arrest of two hours or longer (for transport and repair), followed by delayed resuscitation, is a major challenge.

Attempts to reverse normothermic cardiac arrest in the civilian sector began actually through U.S. Army MRDC-sponsored research by Safar and Elam in the 1950s. We contributed steps A and B of CPR and brain-oriented prolonged life support. In the early 1960s my associates and I put this together into a system of CP-cerebral-R. This system was endowed with guidelines and applied at community levels.

Epidemiologists, like our colleagues in Seattle, and then subsequently in about 30 other communities in the U.S. and in Europe, looked at the results. Sudden death outside the hospital followed by external CPR attempts, has had dismal results for a variety of reasons. When ideal circumstances existed, namely Uhen, a bystander jumped on the patient immediately with very vigorous external CPR steps ABC, then paramedics restored spontaneous normotension with defibrillation within eight minutes (no-flow time two minutes, trickle flow time less than eight minutes), the

results were quite impressive. Forty percent of such cases left the hospital with more or less intact brains (Category 1 or 2). If they would be worse they would remain in a nursing home. So that is mainly a logistic problem.

In the field, we cannot reverse exsanguination cardiac arrest by merely to do this immediately using external CPR. You have to stop the bleeding, and you have to administer or supply an enormous volume of gland substitute into the person. The medic does not have such volumes available.

In civilian cardiac arrests hour VF fewer than one half could have the heart restarted.

Resuscitability of the sick heart is quite different from that of previously healthy dog hearts. Of those patients restarted, about half die in the hospital thereafter, half of those predominantly from brain problems and the other half predominantly from heart problems. Of the 25 percent who leave the hospital, 10 to 30 percent do so with some brain damage.

In U.S. cities the mobile ICU ambulance response times average 8 to 10 minutes. Tbis makes us then not be surprised that in the absence of very vigorous bystander CPR there are such poor results. Extending the longest normothermic cardiac arrest period which can be reversed to complete recovery with good cerebral function, from 5 minutes to 10 minutes, would be a breakthrough. We in Pittsburgh have achieved this in dogs.

Some improvement in clinical outcome data is now being achieved through the introduction of automatic external defibrillation into EMS systems. This can be used by first responders. Epidemiologic data are confirmed by our large-scale clinical study mechanism (of 20 groups in seven countries) which we initiated in 1979. It's name, the brain resuscitation clinical trial (BRCT) is a misnomer. It is a clinical death and CPCR study mechanism. Unique features

include: an enormous data base, highly computerized, of about 4,000 sudden-death cases (majority pre-hospital arrests) estimated insult times; standardized treatment protocols; and outcome measured up to six months. We modified the Glasgow outcome categories (1-5) and separated cerebral from overall performance categories i.e. CPC and OPC 1 (best)- 5 (worst).

At the other end of this spectrum is the molecular level. My associate and friend, Dr. Bircher, points out that DNA -- RNA does not make protein if there is no blood flow. So we have to be a little cautious in our enthusiasm for the in vitro studies.

Another caution, of course, is about the use of rats. We have several examples where certain treatments tried after term proving ischemic arrest of the circulation to the forebrain of the were effective, but when tested in higher animals with clinically realistic cardiac arrest and

intensive care life support, we could not reproduce improved outcome.

The outcome studies on cardiac arrest in large animals, we delved into already in the early 1970s. Dr. Moossy, now in retirement, a renowned neuropathologist, and I decided: Why climb laboriously up the phylogenetic ladder? Why not go straight to the top? So we started with monkeys. Then we watched monkeys make love, and we decided we cannot shorten their lives anymore. So we continued using dogs, one level below. We now have rates on over 1,000 outcome experiments of severe insults in dogs

We have models on different arrest insults, (exsanguination is one), and different methods for restoration of spontaneous circulation (ROSC), such as external CPR (borderline effective), open-chest CPR, and emergency cardiopulminary bypass (CCPB), an experimental tool.

CPB proved significantly superior standard external CPR-ALS to reverse cardiac arrest for heart and brain.

One must support extracerebral organ systems in a standardized way and measure brain and overall outcome through at least 3 days in different ways. The post-ischemic anoxic encephalopathy, known since the early 1970s, will not mature until about three days after reperfusion.

Animal intensive care programs are expensive. It has to be a standardized approach with a very consistent team. Each experiment, if you take all the salaries added into it, costs about $5,000. But you end up with it still being less expensive than randomized clinical trials.

We learned that at least ten goals should be pursued to make outcome studies in large animals reliable. When we see some of these 10 goals not met, we do not trust the results. Each one of these 10 items influences outcome, besides the effects of the treatment to be tested.

Outcome is measured by a modification of the Glasgow outcome scale 1 through 5. Recent studies in Moscow by Gurwich, and in Miami by Dietrich suggest that one should extend this nursing care far beyond one week. There are things happening in the brain even beyond one week, including a further permanent loss of neurons where we did not appreciate earlier; and on the positive side, relearning some function.

For previously healthy dogs hearts, the limit for permanent recovery of good cardiac output in our studies has been 20 minutes of normothermic cardiac arrest. That fits the molecular and cellular data by Jennings and others on focal myocardial ischemia. Even shorter periods of cardiac arrrest between 10 and 20 minutes of no-flow, were followed by scattered macroscopic necroses in the heart. About 5 percent or less of the surface of the heart, inside and outside, will be necrotic We do not know the various

mechanisms behind these scattered necroses. You can speculate on electric injury, ischemia, catecholamine effect, and others.

After 10 minutes of normothermic no-flow or longer, restarting the heart with external CPR becomes more and more difficult. When we insulted dogs with 30 minutes of normothermic no-flow (VF) and reperfused with CPIS, we could restart the circulation with CPIS, defibrillate, and achieve temporarily effective heart beating. But in one to three hours, these hearts fell apart from what seems to be hemorrhagic necroses perhaps due to reperfusion injury.

We studied normothermic no-flow times of 0 to 30 minutes. When the dogs were resuscitated standard external CPR, reliably after more than 10 minute arrest. Emergency CPB so far used as an experimental tool, we are noe taking into the emergency room for CPR resistant cardiac arrest cases.

For cerebral outcome, we looked at numerous factors which might be behind the scatter of outcomes for performance of the organism. With the same no-flow time of 10, 12 or 15 minutes, those drops which were accidentally mildly hypothermic, had significantly better cerbral outcome. This is protective preservative hypothermia of thirty five degrees celsius. We can get a reproducible severe brain outcome with almost 100 percent resuscitability with jump-start bypass.

In the 1970s we developed an outcome model for neurologic and histologic quantification. Using a semi-quantitative technique developed by Moossy, twenty brain regions are being scored histologically.

In normothermic cardiac arrest in dogs, the five-minute limit of normothermic arrest reversibility is not quite as pure as we think. There are some pock ischemic neurons in the selectively vulnerable areas. After cardiac arrest of three minutes, essentially clean brains histologically. When we studied asphyxial cardiac arrest, we found that as little as two minutes of no-flow, preceded by asphyxiation, was enough to give this kind of histologic damage.

equally injurious as ventricular fibrillation arrest. We had hypothesized that it would be less injurious and asphyxiation more injurious.

We found a good correlation between the final neurologic deficit score and the histo-pathologic damage score.

We also have a rat model of asphyxiation cardiac arrest, with outcome over three days.

Since the 1970s we have hypothesized that the multifactorial pathogenesis of the cerebral post-resuscitation syndrome requires multifaceted treatments.

The first such treatments we tested were barbiturates loading, which exerts multiple effects; cerebral

blood flow promotion; our barbiturate therapy and hypothermia.

In the handout which you received, at the back there is a table in which we tried to match derangements to treatments for suspended animation. This table is for protection - preservation - resuscitation, derangements on one side and potential treatments on the other.

On reperfusion, our Drs. Lind and Snyder, in 1971, were the first to document, after total circulatory arrest in dogs of 15 minutes, the CBF hyperemia- hypoperfusion sequence. What is still unknown is what happens, a very delayed secondary hyperemia associated with worse outcome. That's something to go into further. What people thought was so important, the initial no-reflow phenomenon, when we measured CBF by stable xenon computed tomography technique, to go after multifocal CBF, we found that there was diffuse hyperemia during normotensive or hypertensive reperfusion. There was no evidence of a no-reflow phenomenon. With hypotensive reperfusion, however, there were trickle flow areas So we are not worried about the no-reflow phenomenon after 10 to 20 minutes of no-flow in dogs, but we are worried about the protracted hypoperfusion. But we can abolish these trickle-flow and low-flow loci with hypertension and hemodilution.

Metabolism is not matched with low CBF. The cerebral venous PO2 values after prolonged cardiac arrest (12 minutes, no-flow) are dangerously low at times, 10 - 20 minutes Hy. So there are things going on which are not just matched, but the CMRO2, which seems like being in a metabolic silence immediately after reperfusion, later exceeds baseline values while global CBF is half normal for protected periods, up to two days.

COLONEL BELLAMY: Are you going to tell us why? dissociation between flow and oxygen uptake?

DR. SAFAR: This is not exactly known, but there are probably vasospasm, sludging out, endothermal (astrocyte) edema

On the recovery of oxygen uptake we would expect it to go higher than normal, as it has to repair things.

Now, let's assume reperfusion is perfect. There still are chemical cascades which are horrendousIt is important to separate what happens during ischemia from what happens with reperfusion; also what happens inside versus outside cells; and also what happens inside versus outside vessels. It is important not just to say here is one chemical reaction in the whole brain and treating it is going to be the solution of the problem.

A key question to me, a naive clinician, for this suspended animation thrust, is this: I have not found, in spite of the good work of Dr. Garcia, one of our cherished histologists in the field, what happens to ultra-structure in

neurons and other cells when you do not reperfuse and let pan-organic death go on for hours. In other words, needed is a systematic study from total circulatory arrest to time becoming pea soup. Primary necrosis.

DR. SIESJO: It has just been done.

DR. SAFAR: Please give us the reference.

COLONEL BELLAMY: Dr. Virmani may know about the heart. Can you say something about what happens in ischemic myocardium that is not reperfused?

DR. VIRMANI: Necrosis, essentially--

DR. SAFAR: Yes, but how fast?

DR. VIRMANI: It's been shown--that for irreversible injury to occur the artery needs to be occluded for a minimum of twenty minutes.

DR. SAFAR: Those--

DR. VIRMANI: Yes, 40 minutes--it starts at 20 minutes, and you see the early--subendocardial necrosis, then at 40 minutes it extends toward the epicardium and by 6 hours the infarct is essentially transmural, endocardium to epicardium.

DR. SAFAR: When you study an assumption of loss of viability, can never be restored.

DR. VIRMANI: It starts in the endocardium and goes on to the epicardium. The longer you keep occlusion, the further it extends from the endocardium to the epicardium.

DR. SAFAR: There are lots of sub-questions, of course.

DR. VIRMANI: Yes.

DR. SAFAR: For example, if you have this enormous flux of water with calcium and sodium going in, mitochondria rupture.

DR. VIRMANI: Right.

COLONEL BELLAMY: That's occurring within an hour.

DR. SAFAR: Do they rupture before you reperfuse, or do they only rupture after you reperfuse?

DR. SIESJO: In the brain, if you have total ischemia, the amount of water and sodium and chloride and calcium available in the extracellular fluid is very small. And it seems on electron micrographs as if the tissue is more or less frozen until you reperfuse.

DR. SAFAR: So you don't have lethal swelling and rupture of mitochondria until reperfusion.

DR. HOCHACHKA: In total ischemia.

DR. VIRMANI: In total ischemia. That is correct.

DR. SIESJO: If you have incomplete ischemia, it's completely different.

DR. SAFAR: Now, to Dr. Bazan's important contribution, chemically, not morphologically but chemically, during no-flow, without reperfusion, lactate levels off, pH levels off, free fatty acids do not quite level off, but keep going up. Do they eventually level off?

DR. BAZAN: They do, after 30 minutes of complete--

DR. SAFAR: It has to be looked at beyond 30 minutes.

DR. BAZAN: Right. In fact, when you were planning our laboratory many years ago, a model with you--

DR. SAFAR: Yes.

DR. BAZAN: --visited that and found similar changes. They keep going up slowly because at the time that the intracellular pH drops, other phospholates are activated. So the critical ones are the ones that disappear in the initial phases.

DR. SIESJO: The chemical freeze that you have in the brain during ischemia applies to most of the reactions we are discussing here, but not--

DR. SAFAR: Not to the--

DR. SIESJO: --to the increase in the free fatty acid concentrations.

DR. SAFAR: Not to destructive processes. Some still go on.

DR. SIESJO: But the point is that even if your free fatty acid concentrations continue to increase for 30-60 minutes and even though you deamylate and dephosphorylate AMP so that you accumulate adenosine, indocine and hypoxantin, et cetera, this is not destructive until you start reperfusion.

COLONEL BELLAMY: There must be ion shifts going on during that period. Even though there's no change in the total water content of the organ, there still must be ion shifts going on.

DR. SIESJO: No. Actually, you shift the ions to the more or less permanent state within the first five minutes, and then there is no more--there is no way, because there is equilibrium after that, passive equilibrium for sodium, chloride, calcium, and potassium.

COLONEL BELLAMY: In five minutes you have the same ion concentration across the cell membrane; in that right?

DR. SIESJO: Yes.

DR. SAFAR: Calcium entry blocker treatment immediately after reperfusion from 10 to 20 minutes cardiac arrest seems to improve things but does not make the brain normal. Many, many drugs which were tested in the system for

reperfusion therapy, were ineffective. They included combinations of free radical scavengers.

So far only hypertensive reperfusion our mild hyperthermia did improve outcome.

Clearly, in four consecutive systematic outcome studies in dogs, we showed that mild early postarrest hypothermia improves but did not normalize cerebral outcome after 12 minutes of normothermic no-flow. In Study 3 we found that although the lower the temperature for protection-preservation, the more mitigation of brain damage when after normothermic arrest, reperfusion is with deep hypothermia, the brain was worse off. So there is some problem with very low temperatures post-arrest.

Simultaneously and independently, using the rat forebrain ischemia model, three other groups found the resuscitative post-arrest beneficial effect of mild, meaning safe, hypothermia. Mild, 34 degrees, human casualties can reach spontaneously by exposure.

Mild hypothermic treatment post-arrest, however, may not work if it's initiated delayed. There are various beneficial mechanisms by hypothermia.

The hit for the clinic is this, our fifth dog outcome study with mild resuscitative hypothermia (in press in stroke). After 11 minutes normothermic cardiac arrest, we combined cerebral blood flow promotion after cardiac arrest with mild hypothermia for 12 hours and found --at 96 hours-- histologically almost clean brains and normal function in six out of eight dogs.

Comparing this with recently done historic data of only hypothermia or only CBF promotion, their best histologic damage score was still worse than the worst histologic damage score in this study. So we had a pretty convincing suggestion here that the combination of these two approaches -- flow promotion and mild hypothermia, are the best we have to offer at the moment for clinical probability tries. On the drug side -- disappointment. When we added to this treatment of mild hypothermia plus CBF promotion, a combination of seven inexpensive drugs which would hit at these cascades it made outcome worse, maybe through side effects inherent in some of these drug combinations.

We have to find ways, short of a breakthrough discovery, to dissect this with a tedious process, which is very expensive.

How to actively cool clinically is a long story in itself. There are various approaches to internal and external cooling.

It is possible (as has been suggested in rats by Dietrich of Miami but not in higher animals yet) that hypothermia merely delays the inevitable loss of neiurons.

You may end up with an ultimate neuronal loss, anyhow. It still can widen the time window for other treatments.

On the positive side is a study from Dr. Siesjo's lab, showing that cooling rather late after reperfusion may do some good to the very delayed reactions, to which we or others so far have not paid enough attention.

For the battle casualties and suspended animation: low volume hypotensive fluid resuscitation in during uncontrolled hemorrhage opens an opportunity for hibernation studies, not suspended animation. I want to separate these two low-flow with protracted shock, we want to prevent cardiac arrest.

Hypothermia helps doing this during limited-fluid resuscitation, or helps prevent delayed death from multiple organ failure, as we have seen recently in our rat UHS model. Once there is cardiac arrest, this brings usto research into suspended animation question. My defintion is a little more wordy than Dr. Bellamy's. For suspended animation with profound hypothermia we need cardiopulminary bypass (CPB). Bypass is not available in the field, but might be available in the field hospital in the form of portable bypass. That is the studies done by Dr. Tisherman. My friend, alumnus, colleague, our associate, Sam Tisherman, is a very scholarly trauma surgeon who has worked on suspended animation with our dog outcome model since 1988. He has been working with our group since he was a medical student in the early 1980s. Our bypass-dependent hypothesized approach in the dog outcome model is to be induced by something a medic can use without bypass -- a pharmacologic- chemical induction approach. This is the challenge for this group.

COLONEL BELLAMY: Exactly the challenge for the group.

DR. TISHERMAN: Thank you, Dr. Safar. I thank the organizers for allowing me to participate here.

What I'd like to do just briefly is to go through what we've done in the lab with our dog studies on what we have termed therapeutic hypothermic circulatory arrest or suspended animation.

By way of introduction, we all recognize that many trauma patients, both civilian and military, died early without resuscitative surgery. Some of them also arrive alive, but then die in the operating room. There are cases where potentially the use of therapeutic hypothermic arrest could allow repair of otherwise lethal injuries. So we have postulated rapid induction of therapeutic hypothermia for preservation will allow repair of otherwise lethal injuries in a bloodless field, and this certainly could be applicable to other fields of surgery where procedures that are elective procedures could be done under hypothermic arrest.

Our dog model includes the use of hunting dogs. They're anesthetized under halothane nitrous oxide without paralysis, sterile cutdowns, replaced bypass hands, this is

closed-chest, cardiopulmonary bypass, and we monitor various temperatures as we do this.

This is the diagram of the model that Dr. Safar just showed you, but to go through it in a little more detail, the model consists of a period of hemorrhagic shock, which for the first group of studies that I'll talk about that period of shock was 30 minutes. The last study that we did, we extended that, and I'll get to it. The mean pressure of 40, and this is at normothermia, the temperature line. After the period of hemorrhagic shock, we then placed the animal on bypass and cooled him down as rapidly as possible. This procedure takes about 20 minutes, bypass cooling, seeing the temperature come down. The goal is to get the core temperature down to about 10 degrees Centigrade and get the brain temperature that we're monitoring by tympanic membrane temperature down to at least 15 or below. That takes about 20 minutes.

During that time, in our initial four studies, we also hemodiluted the animals, and I will mention later on about whether or not we should have been doing that. But we diluted them down to a hematocrit of 5 percent or so. After they're cold, we then stop the circulation completely for a period of one hour to two hours. In some animals, we have looked at profound hypothermia where we allow the brain temperature to drop even further. It goes down to about 5 to 7 degrees. In other ones, we've maintained the tympanic membrane temperature at 15.

After the period of total circulatory arrest, we then reperfuse, again using bypass, which is continued for a period of two hours. They have a cannula in place for 24 hours and they're observed for 72 hours. So it's a 72-hour outcome.

Just like some of the other studies Dr. Safar mentioned, we look at the overall performance category, OPC1 being normal, 5 being death. We looked at neurological deficit scores. At the end of the study, we did perfusion for six and sacrificed, a complete (?) and brain cystology.

Again, the model of (?) shock, 30 minutes for mean at 40, bypass cooling, therapeutic hypothermic circulatory arrest, and then intensive care for 72 hours.

Our first study was just to kind of get some idea using this model what was the safe duration of hypothermic circulatory arrest under deep hypothermia, and this is what is clinically used for cardiac surgeries and neurosurgical procedures also. We looked at 60, 90, and 120 minutes of arrest, and we found that 60 minutes was safe. Now, safe is kind of a relative term because here safe, we are saying in terms of neurologic function. At 60 minutes, they all walked around and acted like normal dogs. One foreign factor is that they all did have some histologic changes. So that's something that we need to work on, and I'll get into a little more of that later.

Let me go into a little more detail on some of these other studies.

Next we thought, okay, if cold is good, maybe colder is better, and we did a study looking at deep hypothermia versus profound hypothermia. Again, deep is 15 degrees, and profound hypothermia, we dropped the brain temperature down to about 5 to 7 degrees. We found, looking at the functions, that the functional outcome was significantly better with profound hypothermic arrest. In this study and in the next three studies I will mention, we are using a model where we use two hours of circulatory arrest after a hemorrhagic shock. So all I know is that it's the same period of two hours of arrest.

Now, these are the same two groups. The next thing we did was to compare this with adding, in addition to the deep or profound hypothermia, the use of a preservation solution. At that time, this was around the time that the University of Wisconsin solution was being used actively for--it still is being used, but this is shortly after it was developed--for preservation of organs for transplantation. Since this is so good at preserving other organs, perhaps it would be useful for preserving the brain during a period of hypothermic arrest. We actually found that it didn't do a very good job. We looked at it both at deep hypothermia as well as at profound and really had very poor results.

The histologic damage scores corroborated very well what we found functionally, that the best results were found with profound hypothermia, just using hemodilution--and hemodilution is done with crystalloid solution, just plasmalyte. The use of the University of Wisconsin solution really had no benefit histologically, either.

One of the other key questions regarding this use of hypothermic circulatory arrest and the use of bypass for the trauma patient is, in general, bypass is used with heparin for completely systemic anticoagulation. Well, we wanted to see, can we get the same kind of results if we don't get any systemic anticoagulation? Can we use a bypass system that's totally heparin-bonded so that we don't have to use the heparin?

There are questions that perhaps heparinization would be good for the brain, might not be good, so that was one of the other aspects of this. What we found was that we could so this experiment just as easily without giving systemic heparin and using a heparin-bonded bypass system our results were exactly the same as they had been without the heparin-bonded system.

I'll mention one other study that was done about the same time was the question of the hemodilution is something--there are a lot of aspects of this that really have to be looked at in detail. One of the things was hemodilution, and when we looked at it, we did a small series of several animals using a higher hematocrit during the period of circulatory arrest where we kept the hematocrit around 18 to 20. And the suggestion, anyway, from the small

series that that's better than the severe hemodilution that we used. We thought that the severe hemodilution was useful because of improving micro-circulatory flow, decreased the sludging, but, again, that's something that really needs to be looked into with more detail.

What I want to spend a little bit more time on are the results of the last study that we've done. This study was done to determine the limits of normothermic hemorrhagic shock, treated with standard fluid resuscitation that would allow long-term survival in dogs. This hasn't really been detailed all that well. Then to determine whether the addition of profound hypothermic circulatory arrest for 60 minutes would increase morbidity and mortality. So if we take hemorrhagic shock insult, that's the limit that a dog can tolerate, and then add on top of that an hour of hypothermic circulatory arrest, what would that do to the animal?

Some of the pilot studies that we did to kind of get some handle on this limit, we took a number of dogs, subjected them to 90 minutes of normothermic hemorrhagic shock at a mean pressure of 30, which goes back to some of what was mentioned earlier, that this mean pressure of 30 seems to be a very significant limit for the brain and for the rest of the animal. We did three dogs with standard resuscitation and two with hypothermic arrest. Two out of three dogs with standard resuscitation survived but remained ventilator dependent. All the others had initial recovery and deteriorated, and basically by 30 to 38 hours all five of these animals had died. So 90 minutes of shock at a mean of 30 is not survivable, at least in our hands, in dogs.

Then based on that pilot study, we backed off a little bit on the duration of hemorrhagic shock, and we did a more definitive study looking at 60 minutes of hemorrhagic shock, either with a mean pressure of 30 or a mean pressure of 40, and then in Groups 1 and 3, they received normothermic fluid resuscitation, the standard fluid resuscitation with return of shed blood and lactate Ringer's solution, or in Groups 2 and 4 we then cooled them down and cooled them to profound hypothermic level. The brain dropped to around 7 to 10 degrees, and had a period of circulatory arrest for 60 minutes in both of these groups, then the standard bypass rewarming and resuscitation and long-term outcome as in our other study.

Now, what we found was that looking first at survival, the 72 hours, there were five animals in each group, and the two groups that had a mean pressure of 30, whether or not they had standard fluid resuscitation or had the addition of one hour of profound hypothermic circulatory arrest, only two out of five of those animals survived for 72 hours. In the groups that had a mean pressure of 40, they all survived whether or not they had standard fluid resuscitation or had additional insult of one hour of profound hypothermic circulatory arrest.

Very importantly, and this minimal here really is about 0. These brains were quite clean; no matter whether the dogs had profound hypothermic circulatory arrest or had standard fluid resuscitation, the brains were clean. So 60 minutes of profound hypothermic arrest, even after a period of hemorrhagic shock, at those levels of temperature produced clean brains. But the rest of the animals' organs had problems. The dogs that died, they had pulmonary edema, evidence of pneumonia, hemorrhagic enteritis, which was very severe in some of them, petechial hemorrhages throughout many of the organs.

In summary, from this last study, we found that 60 minutes hemorrhagic shock at a mean of 40 allowed survival. If we dropped the pressure down to a mean of 30, the mortality increases 60 percent. Ninety minutes of shock was basically not survivable, no matter what. The important thing is that the addition of one hour of hypothermic circulatory arrest did not alter outcome, and the deaths were the result of delayed multiple organ failure, not neurologic damage. So our feeling is that this is technically feasible. Whether it's something that's feasible in the field needs to be worked out, but it's something that feasible and could become part of our armamentarium for the patient that has been severely injured, is in severe hemorrhagic shock, and certainly has the potential of trauma survival, a victim of trauma who otherwise would not survive.

COLONEL BELLAMY: It's my personal view that hypothermic cardiopulmonary bypass is the essential component at the hospital level of any such approach. I think what we see here mostly shows that. I would question the scenario. If we want to talk about scenario development, my personal view is that I'm not sure how often people go into a low-flow, prolonged hypotensive state following trauma.

Dr. Champion, do you want to comment on that? The data I see in the combat casualty care data base is that, casualties have an injury, they bleed out rapidly. They don't go into a fixed-pressure state for very long. Maybe Dr. Champion wants to comment on this.

DR. CHAMPION: Well, I think you're probably right in general, but, you know, with Maddox's work about non-resuscitation and so forth, there probably is some sort of steady state being reached, when we're talking about hypotension, to 90 systolic, which is not--

COLONEL BELLAMY: It's 130 or 140.

DR. CHAMPION: Right. There's a big difference between resuscitation, no resuscitation that Maddox has been talking about, and also that's been written up. So I think there is an opportunity for some sort of steady state, but it's how to identify those patients and how it's reached I'm not quite sure.

COLONEL BELLAMY: I can imagine you doing studies in the future where, in fact, you exsanguinate the animal, let the animal maintain a state of cardiac arrest for 10

minutes or 15 minutes, and then see to what extent the modalities you have studied here are applicable.

I also think it's very interesting that the cause of death frequently was multiple organ failure and not neurological dysfunction. We have been focused upon brain injury, of course, but obviously there are other parts that have to be cleared as well.

Any comments?

DR. SIESJO: The question regarding, so to speak, the scenario.

COLONEL BELLAMY: Yes.

DR. SIESJO: How many of the patients you are focusing on now would have a cardiac arrest? And how many will still have a functioning heart but very low blood pressure?

COLONEL BELLAMY: The data that I can see from the Vietnam data base would be something on the order of 100 of the casualties would sustain exsanguination cardiac arrest within a matter of 5 or 10 minutes. Some at the tail end live for as long as an hour before they reached the hospital. Roughly 80 casualties would reach the hospital within an hour, who would, in fact, be bleeding and would be in circulatory shock. About 10 percent go on and die. Unfortunately, the common scenario is that most casualties are going to exsanguinate very rapidly.

I think your findings are very important. I think normothermic cardiopulmonary bypass would be an essential component of any resuscitative strategy, but the question is, as you say, the scenario development. I think, unfortunately, the scenario is a typical one. People just exsanguinate, bang, a hole in the pulmonary vein, for instance.

DR. SAFAR: If we assume this is what happens, more often than we realize, also in non-military situations, how would the paramedic decide whether he should prevent cardiac arrest with hypothermic limited fluid resuscitation (where the ideal fluid for low volume resuscitation, which he can carry has not been worked out yet

to prevent cardiac arrest -- or to kill him with suspended animation when he still has a thready pulse?

COLONEL BELLAMY: Yes, exactly right. I think the ARPA advanced technology that I alluded to in the beginning of my introduction may provide some hope of resolving that question.

MAJOR BRUTTIG: Let me jump in for just a minute and confuse the issue a little bit more, because some of what we see, some of what we refer to historically in terms of defining the problem, I think can be really laid at the feet of the model.

COLONEL BELLAMY: Could be what?

MAJOR BRUTTIG: Laid at the feet of the experimental model.

COLONEL BELLAMY: That's the problem with shock research during the last 70, 80 years. It has addressed problems that clinically are not important.

MAJOR BRUTTIG: Right. And what is exciting about what Dr. Tisherman and Dr. Safar are presenting here is that in some cases they have something approaching the Wickers' model. In other cases, they had an uncontrolled hemorrhagic shock imposed on that.

Now, what I'd like to do is depart from that for just a minute and talk about uncontrolled hemorrhagic shock without any preimposition of a Wickers' type hemorrhage or whatever.

In those studies--and they predated the studies that you were talking about with the medics, which was a human trial. These were the animal studies that set that up. What we were looking at was, in a large animal, what happens when you have a relatively devastating uncontrolled hemorrhage from a large vessel? Say we tore a hole in the aorta and identified a range in which we could get a survivable outcome. That happened to be associated with about a 33 percent bleed; roughly a third of the blood volume is lost. Total volume equivalent is roughly the volume equivalent that Dr. Bellamy reports in terms of those combat casualties that die. These were pigs of about 45 kilos, and death was associated if the volume exceeded about 700 or 800 mL on the average. But these animals typically would lose about 500 or 600 mL, and so the equivalent is, if you go over a liter in a short period of time, you might have death in a human.

Once we've identified the size of the hole, we decided to do these in conscious animals, which is unique, I think, among the experimental designs for uncontrolled hemorrhage, because we wanted to get away from the effect of anesthesia. And so we chronically instrumented these animals, and then caused the vascular deficit.

Now, these animals were trained in the lab, so we weren't worried about imposing some sort of psychological, if you will, or emotional trauma on the animal, but came in, got baseline data, and pulled the rip cord, if you will, and caused the hemorrhage.

What happens is, because it really falls on what Dr. Siesjo was talking about early on, is that arterial pressure goes to 30 mL of mercury in something less than a minute, which you would associate with reasonably quickly following central nervous system deficit. Okay? And you've laid the experimental evidence very elegantly.

These animals essentially went into some unconscious state for a short period of time, and without prolonging the issue, by 15 minutes every animal was back on its feet and behavior was normal.

COLONEL BELLAMY: I'm sorry. Was it open vessel?

MAJOR BRUTTIG: With open vessel.

DR. SAFAR: On his own?

MAJOR BRUTTIG: On his own. With no resuscitation fluid, et cetera.

The point is that when we looked at this, we looked at peripheral vascular resistance as when we calculate peripheral vascular resistance from a limited amount of cardiovascular data. And peripheral vascular resistance drops. It drops enormously.

If you conduct a Wickers' hemorrhage, peripheral vascular resistance rises dramatically. So we began to think that maybe some sort of event, whatever that event might be, is a reflex event to decrease flow out the hole, the hemorrhage, by dropping peripheral vascular resistance.

Now, we don't know whether it's that or it's some artificial manipulation that occurs in the calculation of peripheral vascular resistance. The point is that very low pressure didn't cause death, nor did they appear to cause cerebral deficit. And we followed these animals out for about two weeks.

DR. LEWIS: They didn't plug up the hole?

DR. BRUTTIG: What happens is that when the animal falls--and it just kind of goes into a reclining position, not over on their side, but they go down like a dog lays down and whatever. There's pressure on the abdomen, so you get a tamponade of the hole. There's time for clotting to occur. And that is kind of important. When the animal gets back upon its feet, its pressure is no greater than 50 mm of mercury.

DR. CHAMPION: So it does go up? It goes up to--

DR. BRUTTIG: After two hours, it's 55 mm of mercury.

COLONEL BELLAMY: We have to move on. This is fascinating, and we're going to ask you questions about 3 o'clock. Of course, the scenario development is very important because otherwise you end up with an answer to a question that doesn't exist. We need to go to Dr. Bazan.

DR. BRUTTIG: Sure. What I'm getting at, the point is that physiologically you can tolerate very low pressures if you look at the total situation here. We're not talking about anesthesia. We're talking about spontaneous recovery. We're not talking about fluid resuscitation. So sometimes we confuse the issue.

COLONEL BELLAMY: Dr. Bazan will try to summarize what we've just heard and give his own ideas.

DR. BAZAN: That will be quite a difficult task. I'm at LSU in New Orleans, and we have a laboratory focusing on brain damage, and we do things with models of ischemic damage. We have a number of models, usually, but what I

would like to do is share some of the ongoing work at the end in the last few minutes, but begin by trying to highlight in the first 10 minutes some of the central issues brought up earlier in the morning. I will try to highlight some of the points of the beautiful talks given by Bo Siesjo and John Hallenbeck. I'm not going to make much of the summary of the last two very nice presentations.

I think this is a critical experiment that tells us something new about the windows to treat head injuries, because we have thought that pharmacologically one can intervene to limit damage before this critical event at shorter time periods. I was very impressed by the fact that PBN, this free radical scavenger, in fact, even given before--and this is an experiment where there are two hours after recirculation and four hours recirculation it is active for seferic lactate accumulation both in the penumbra and in the focus. And I think it's remarkable, the effect in both areas.

The other item is just try to--here is ATP which is also remarkable from the point of view of almost restoration to control levels. So this is telling us that very critical mechanisms have been effected way beyond limiting the free radical accumulation. The consequences of the free radicals actually on the mitochondria function have actually led to this spectacular restoration of ATP.

DR. CHAMPION: When was the drug given?

DR. SIESJO: The drug was given one hour after circulation.

DR. BAZAN: Right.

DR. SIESJO: So the two hour and four hour, then you have two groups.

DR. BAZAN: Right. This is sort of a summary of Bo Siesjo's concept of the two therapeutic windows, and I think it's important to remind us of what he told us early this morning that the first window is narrow, it's reflecting calcium influx and the triggering of calcium-related events, related to little production of mediators, inflammatory cytokines and free radicals. Once this series of events has been triggered, glutamate or calcium antagonists can no longer stop the grass fire. I think this is a very important concept.

The second window of opportunity is large, three to six hours, or longer, because certain drugs can block the reactions solicited by the initial cascades. These reactions either encompass the upregulation of adhesion molecules for PMNs or prevent secondary mitochondrial failure.

I hope Bo would say I somehow do justice to all the concepts he brought us in your very nice lecture.

I was very struck by the lecture of John Hallenbeck and by the thinking behind this approach. I think this is a very important experiment where, in spite of the fact that the red blood cells are maintained during hibernation, there

is a selective and remarkable diminution in platelets, in neutrophils, lymphocytes, and trace amounts of monocytes.

One of the interesting possibilities of this approach is highlighted in this experiment, and that is the point that John makes so elegantly, that this model may allow the discovery of unknown mediators and of unknown mechanisms, and perhaps more importantly, this type of model may allow us to try to understand how these networks of mediators promote such injury.

I think this is a remarkable effect here. ICAM-1 expression is a critical player, as you know, in ischemia reperfusion mediator damage to many organs, and there is something here that is actually hidden in this experiment and perhaps will be very important in the understanding and identification and discovery of new endogenous mediators. I think it is a very interesting and powerful approach.

What I would like to do in the next 20 minutes or so is to bring some of our thinking as well as a sort of summary of other work going on in this area, and I would like to emphasize endogenous neurotoxic mechanisms, and in a few minutes I would like to emphasize endogenous neuroprotective mechanisms, because I think this is one approach that will lead us to identify pharmacological means for the stabilization in head injury.

This list of neurotoxic substances, in fact, endogenous neurotoxic substances, is divided into intercellular mechanisms and intracellular mechanisms. The intercellulars are the excitatory amino acids, neurotransmitters. The intracellulars are consequences of hypoglycemia, hypoxia, at the level of excitable membranes, the phenomenon of membrane perturbation. The growing evidence that amyloid peptide and many other peptides that occur normally in the brain are actually central players of neurotoxicity, the amylegenic peptide, not only, as you know, Denn and Silko have demonstrated in a very elegant paper recently that several of these peptides are produced normally in the brain, and they accumulate beyond certain levels in diseases such as Alzheimer's. The reactive oxygen species are also intercellular neurotoxic substances. There are a number of other factors like thrombin and the very large and growing list of bioactive lipids, the latest platelet-activating factor which, of all the lipid mediators, is the most open neurotoxic bioactive lipid.

Then intracellular, calcium, that will be a failure in the activation of endonucleases. Bo Siesjo emphasized this point, and as a consequence, in the triggering of apoptotic cell death, reactive oxygen species, proteases, phospholipases and kinases.

Now, let's go out of the neurons for a moment and let's think about mechanisms that are important from the point of view of endothelial cells, and this is a nice cartoon because--a nice outline, because it brings into perspective the complexity of mediators, as John Hallenbeck stated. Here we are talking about the role of endothelial

cells in ischemia reperfusion injury, and this is an oversimplified summary where in the top free radical production and lipid peroxidation will lead to endothelial cell injury, but then you have groups of mediators--nitric oxide--here it should say endothelium, all the bioactive ecosinoids, specifically thromboxins, that will be increased, protacycline is decreased, thromboxin B4 is increased and PAF is increased. The complement activation proteins, all the cytokines, the expression of adhesion molecules.

I just want to emphasize that we know very little about which are the real mediators. We are actively embarked on a program characterizing a new ICAM, the ICAM-4, which seems to be blade specific, and this is a totally new adhesion molecule that we knew nothing of up until now. It has just been cloned in collaboration from our laboratory with a group in Seattle. And here there is nothing about chemokines. The expression of chemokines in the brain and the acute changes of chemokines in ischemia, in the early phases of ischemia, is a very remarkable phenomenon. I will not have time to talk about it, but I just wanted to mention it. If somebody would like me to address that issue, it is an area of intense interest.

Now, on the other side of the coin, there are also endogenous neuroprotection mechanisms linked to signal transduction, to normal neuronal or neuro-signal transduction. And I think this should actually allow us to see in strategies for pharmacological stabilization, because some of them are mechanisms that are very rapid, that occur within minutes, and then there are others which are delayed endogenous neuroprotection mechanisms. Those very rapid ones are phosphorylation, the phosphorylation of ion channels. They are very rapid changes in cytoskeletal regulation of ion channels. There is a change happening within minutes at the level of redox modulation of receptors such as the NMDA receptor, and it's a very new mechanism to regulate NMDA receptor functions.

There is a recently cloned enzyme, pafaseta hydrolase, that is extraordinary in reaching the brain. In fact, there are several pafaseta hydrolases in the brain, and this enzyme is a very powerful regulator of the concentration of platelet-activating factor in injury. And receptor modulation, for example, receptor modulation of a new toxic compound like PAF delay, are mainly mechanisms at the level of gene expression where, for example, calcium-binding proteins are in use, and after ischemia reperfusion, it has been shown that three of those genes are actually upregulated as time goes on after recovery. And what the cells are trying to do is, in fact, to generate calcium-binding proteins, but I want to make a point that these genes are immediately early genes, and some of the calcium-binding protein can be upregulated de novo within 30 to 40 minutes. So it's delayed, but as compared with these changes that happen in minutes.

Also, regulation of excitatory amino acid receptor expression, the induction of antioxidant enzymes and against receptor modulation at the gene level.

I would like to make two specific points after these general comments that are linked to cell signal transduction and the accumulation of lipid mediators in ischemia reperfusion.

This cartoon is applicable to all organs, but this kind of a sequence in ischemia reperfusion not only happens in the brain where the expression and activity of the enzymes involved is maximum, but the heart, kidney, and all organs that have been looked at in ischemia reperfusion conditions will display this type of cascade. The central initial player is phospholipase A2 that is activated initially very rapidly at the onset of ischemia and the onset of trauma. What we knew for many years is about free polyunsaturated fatty acids such as arachidonic acid accumulating in the brain within minutes, and we knew that arachidonic acid is a precursor of prostaglandins and lipoxygenase products. Most recently in the mid-1980s, we realized that also platelet-activating factor is made by the same enzyme. And so after ischemia, there is an accumulation of polyunsaturated fatty acids, platelet-activating factor, and there is a disruption in the excitable membrane composition of polyunsaturated fatty acids.

One other key player in the brain and in the heart is a fatty acid with six double bonds and 22 carbons called hexanoic acid, which is also released at the time of ischemia. And so that creates a background of a structural modification to, in fact, set in motion further damage to the membranes through lipid peroxidation, for example.

At this time there is a low availability of oxygen, so all of these reactions beyond the fatty acid are slowed down, but during the reperfusion phase, as Dr. Safar emphasized, I think this is a critical point, not only the formation of active mediators like prostaglandins and lipoxygenase products but also non-enzymatic peroxidation products are accumulated, and damage will occur.

Before going to the next slide, I want to tell you that there is a lot of new knowledge in this area. This is an over-simplified cartoon. We know now that in the brain there are at least five phospholipases A2, and I don't think we understand precisely which phospholipase A2 is a critical player at the synaptic level, which phospholipase A2 is a critical player at the mitochondrial level, because these reactions are also happening at value levels in the brain.

One of the new phospholipases A2--and this paper is not yet published--is a low molecular weight phospholipase A2 that co-exists with glutamate in the synaptic vesicles, and during neurotransmission is released with glutamate into the synaptic cleft. Our laboratory analysts are very actively trying to understand the role of specific phospholipases in the early phases of brain damage.

I'd like to make, as I said, two points briefly. This is an old experiment with a PAF antagonist, and that's how we began putting a lot of effort to try to understand the significance of the accumulation of PAF in the brain during ischemia. These are gerbils, and during ischemia reperfusion they were injected with a PAF antagonist, which is BN-52021. It is a PAF antagonist purified out of a gingko belova(?) extract, and it was the first PAF antagonist to be isolated. The PAF antagonist here was given 10 minutes after the ischemic insult, and there was a spectacular recovery of the cerebral blood flow, and measuring the accumulation of neurotoxic substances, we found that this compound was neuroprotective. This is a paper of about ten years ago done in our laboratory by Tom Paneta and Vito Marticelli.

Then we began exploring the mechanism of action of this PAF antagonist, and what we found is that platelet-activating factor, at least it's very selective action on excitatory synaptic transmission. In fact, I will not show you all the data. I have two of my (?) Klampers, and this is a paper that Gary Clark and Guinio Kato published in Nature recently, actually about a year ago, where the effect of these PAF antagonists was identified. These are two different PAF antagonists, and I will not go into detail into all of these expressions, but what it shows, these are CA1 neurons in the hippocampus and the phenomenon of long-term potentiation.

What this shows is that when a stimulation is given here, there is a sustained activation of the synaptic circuit, and one PAF antagonist, BN-52021, blocks completely that. The other one does not. The controls overlap the top curve. This type of experiment, this paper in Nature and one prior to this that we published in Neuron, demonstrates that the neuroprotective PAF antagonist is active on the presynaptic side. Let me just show you here.

This is actually a synaptic ending. Dr. Hallenbeck isolated these. If you imagine a synaptic ending cut here, it's a synaptosome, what he was telling us with those elegant experiments that he had done. And our electrophysiological data and biochemical data shows that platelet-activating factor activates release of glutamate. So it's a very powerful activator of the release of glutamate, and there is a receptor for PAF in the presynaptic membrane that was identified about five years ago in our laboratory by Vito Marticelli upon which the PAF antagonists act. So the neuroprotective effect of this PAF antagonist is by slowing down the release of excitotoxic glutamate.

In fact, this is sort of a summary of many experiments done in our laboratory because platelet-activating factor as glutamate and as interleukin-1, as many other biologically important messengers is a dual messenger. PAF is a central player in synaptic plasticity because it modulates release of glutamate. But if that process is in any way impaired by an enhanced concentration of PAF, such as occurs in head trauma or in ischemia, then there is an enhanced pathological release of glutamate.

What we know is that, for example, in seizures the phospholipases are accumulated; in ischemia, phospholipases are accumulated at the level of the synapse, and in ischemia reperfusion of the gerbil, we know that this PAF antagonist, as I told you, is neuroprotective. In presynaptic membranes isolated from these synaptosomes, the PAF antagonist displaces (3H) PAF binding, suggesting that it acts at the level of the presynaptic ending. So PAF, by acting on this side, increases excitatory neurotransmitter release, and the PAF antagonist inhibits this side.

What the paper in Nature demonstrated is that PAF is a retrograde messenger in long-term potentiation, but when you break these circles, these normal circles, circuits of communication between cells by this lipid mediator, then neuropathological consequences occur.

DR. HALLENBECK: I probably missed this, but does the PAF increase the release of the excitotoxins presynaptically--

DR. BAZAN: Right.

DR. HALLENBECK: Directly, or is it through effects on the calcium channel?

DR. BAZAN: Well, yes. Our hypothesis is that there is a calcium channel, also there is a synaptic ending, yes. This is not very well understood yet. We think that there is a calcium channel. This is an area of intense activity in our laboratory now, and this calcium channel activates a member of the synaptic family, which allows the synaptic vesicle to be released. The concept is that PAF in a presynaptic enhancer of excitatory amino acid neurotransmitter release is not only a physiological messenger, but participates in ischemic neuronal damage.

You see, many strategies for neuroprotection have been followed by the approach of blocking the post-synaptic receptors. In a way, this system may allow to close the faucet that releases glutamate from the pre-synaptic ending, and I think it is giving us clues into that type of mechanism.

We know also that it participates in seizure generation. For example, we have models of trauma where seizures have been developed, and we have a tremendous accumulation of PAF and all of this mechanism is set in motion.

Actually, I was born in Argentina, and I was invited to write an editorial for Nature that came out in April this year, and the conclusion of this in Nature was that new drugs that affect metabolic PAF-ways might be very useful. And the editor called me, and I said, well, can I get away--it is not a spelling error, you see? It is not a Spanish accent--

DR. BAZAN: And they began laughing on the phone, and it was published in Nature in April with metabolic PAF-ways.

One point before concluding, we really feel very strongly that PAF is a member of a network that engages many other mediators, and we do not understand very well how this happens. But there are two interesting features of platelet-activating factor because the precursor sits in the membrane, which is a phospholipid, but the precursor happens to have a lot of arachidonic acid in this position. So here we have a system that when the signal activates this enzyme, arachidonic acid is released, and immediately prostaglandins and other mediators can be generated, and then PAF is made. So out of the same signal, several mediators can be generated.

The second point, as time goes on, we are unraveling PAF-mediated events, and PAF activates the synthesis of leukotriene C4. It's an activator of its own synthesis and activator of the synthesis of prostaglandin. Our laboratory found in 1989 that PAF is a transcriptional activator of several genes, of immediate early genes, of a new gene called prostaglandin synthase or COX2, activate of metalloproteinases, activate the gene heparin-binding EGF-like growth factor, and urokinase PA.

We are trying to put in perspective the meaning of these actions of PAF in delayed damage and in delayed consequences of injury. PAF is also an activator of PMN, of complement and of oxygen radical formation. And there is a cousin of PAF which are oxidized phospholipids that accumulate very rapidly in ischemic injury, not only in the brain but also in the heart and in endothelial cells.

You see, this is glycerol. Instead of having an alkyl chain here, it has an acyl chain, phosphate and colloid. But in this position, it has an acetate, a two carbon. The oxidized phospholipids have an oxidized acyl chain, a short fatty acid oxidized. And this oxidized phospholipids are very active biologically, almost as potent as PAF itself, and they are just beginning to be uncovered as a cousin of these made during ischemic reperfusion injury.

How long, four minutes, five minutes?

MR. GRAY: Another 10 minutes.

DR. BAZAN: Ten minutes, okay.

Let me show you--I'll move a little bit ahead to--John Hallenbeck made a very important point, and he said that perhaps we don't know all the mediators involved yet. Perhaps some of them have still to be discovered.

In 1991, it was cloned, it was discovered the inducible prostaglandin synthase. Until that time, we thought that prostaglandins, after arachidonic acid is released, were made by a constitutive prostaglandin synthase. Only in 1991, the inducible gene was cloned. And this inducible gene is a very exciting one because it's the site

of action of glucocorticoid. Glucocorticoid have been known for many years to inhibit the formation of prostaglandins, and nobody knew how they worked. And we know that the promoter of this gene has a site upon which glucocorticoids inhibit expression of this gene.

Today we know that many substances like cytokines, EGF, they activate the gene. The gene doesn't exist in the brain under resting conditions. But in ischemia, they all suddenly appear.

This is our own data on kainic acid-induced seizures, but with ischemia, it happens the same.

The point here is in this instance what you see is ours during kainic acid-induced injury to the brain, and you have messenger RNA for this inducible prostaglandin up at one hour and a very high level of three hours. In ischemia reperfusion, in global ischemia, the peak is at one hour.

This is an experiment done on hippocampus of animals under kainic acid-induced seizures. I will not show you the whole story, just one slide. These are Western blots to show that the messenger RNA have been translated into COX-2 protein, into the active enzyme. And here is the PAF antagonist showing an inhibition of these. Our laboratory has found that PAF action, many of the actions that we are talking about, are mediated by the induction of COX-2, and I will show you--you see, prostaglandins are normally made by this constitutive prostaglandin synthase. And in ischemia and in trauma, all of a sudden platelet-activating factor is the linkage to induce the new enzyme that will make pathological prostaglandins.

We knew nothing about this until one year ago, and this is something that occurs in the brain within 5 to 10 minutes--within the first 10 minutes of injury. That's why I think it is so important, the concept that we have to keep--that John Hallenbeck made about newer players in damage.

What this tells us--and I will go to the very last slide. Let me just go because these are on molecular biology, but I won't bore you with that. This is my last slide. It's a little busy, and I will take two minutes to go over it.

What this tells us is that mediators of injury such as platelet-activating factor, in addition to having an action at the presynaptic level releasing glutamate, they elicit effects at the gene level. And the consequences of this, I will go into this in a minute, are either plasticity changes, differentiation during the development, or apoptosis. We know that some of these mechanisms are critical in the decision-making process of whether or not a cell will survive.

What happens here is that our laboratory has identified transcription factors, in fact, the brain injury protein, that seems to be critical in inducing selectively certain genes under pathological conditions. The increases in genes that you see in ischemia represent a mixture of a

defense of the cells and the suffering of the cells that are about to die. But some of those genes can be blocked with antagonists. We have some antagonists that can block some of these responses, so in models of injury we are actually, in fact, seeing the effects and asking the question of whether or not they can be neuroprotective.

What you see here is primary response genes or immediate early genes like C4s. And the inducible cyto-oxygenase or COX-2 is a primary response gene that encode an enzyme. Others encode transcription factors that activate secondary response genes that, for example, encode receptors, that in turn affect ion channels, and the ion channels affect signal transduction pathways that modify target genes to produce other proteins embodied in other responses.

The interesting new element is that the mediator of inflammatory injury like PAF through a gene upregulates prostaglandins which are also mediators of injury. There is a sort of a feedback loop of the inflammatory response that we were not aware that existed in neurons. So it's very much a cellular and a molecular approach to try to understand the mechanism of action of many of these new mediators, what we are embarked on in the last year or so.

I will stop here. Thank you very much for your attention.

COLONEL BELLAMY: Are there questions of Dr. Bazan?

DR. SIESJO: I think this is a beautiful story. I just am trying to make up my mind how relevant this is for ischemia reperfusion. Would it be possible for you to go back to your gerbil data on ischemia reperfusion?

DR. BAZAN: Sure.

DR. SIESJO: The reason why I raise this question is that we have used both these PAF antagonists in rats. There is no effect whatsoever on the brain damage induced by 10 minutes of ischemia. We see no effect whatsoever. But if we could show that signal slide you had there.

DR. BAZAN: Sure. Well, let me go to that. Let me tell you a little bit about it. I think it is an issue with the models, obviously.

DR. SIESJO: That's my point. That's what I want to--

DR. BAZAN: Krigenstein has published two papers confirming in gerbils and showing--and we have seen now in the middle scenario--let me go back to that.

DR. SIESJO: Yes, that's very influential.

DR. BAZAN: Yes, I think it is an issue of the model. That data was on gerbils.

DR. SIESJO: Because if you look at your CBF data, you have a short period of ischemia. And if you look at the vehicle-treated animals, the blood flow is actually

decreasing down to levels of around 10 after 90 minutes of reperfusion. And if you look at your blood pressure, your blood pressure is well below 50 mm of mercury.

DR. BAZAN: Yes.

DR. SIESJO: So it could be that you are looking at ischemia with a low perfusion state afterwards.

DR. BAZAN: Absolutely.

DR. SIESJO: So you're looking at something which is prolonged ischemia in that sense. So my question is: Aren't you affecting the microvessels here? Don't you think that the PAF is really acting on the microvessels?

DR. BAZAN: In fact, that was the point in the discussion. You see, at the time that we did these, we were not aware--at that time we hadn't identified the receptor; we hadn't identified the effect on the release of glutamate. And we said nothing about that. And we said this is either an effect on neurons or an affect on the microvasculature. Your point is very well taken.

Even today, we cannot rule out that that is the case. We cannot rule out that there are multiple effects happening, because very likely--you are absolutely correct--something is going on in the microvasculature under those conditions.

DR. SIESJO: Thank you.

DR. VERMA: I have a couple of questions. One is on the glucocorticoids. In light of this, what you've shown us, can you comment on glucocorticoids having an effect on ischemic injury in the brain?

DR. BAZAN: Thank you for the question. It's an important question, because this is not the only effect of glucocorticoids. The complexity in the data of experiments using glucocorticoids in ischemia and in other forms of brain injuries is due to the fact that, for example, they will affect three genes, and they upregulate the interleukin-1 type 2 receptor, which is a receptor without a signal transduction pathway. It is a decoy receptor. They upregulate the GRGs, the glucocorticoid responsive genes. They upregulate many monokines. So I think the data is controversial with glucocorticoids for many laboratories, and we don't use glucocorticoids in our models because of the complexity of the effects.

Does that answer your question?

DR. VERMA: Yes. The other question I have is: How does one reconcile the observations that you induce genes and proteins and hypoxians with the observations that proteins shut down at the same time? Is there some selective process that--

DR. BAZAN: Right, right.

DR. VERMA: --certain proteins--

DR. BAZAN: Right. You see, the main changes of complete ischemia are induction of immediate early genes, and by definition, immediate early genes transcription is independent of protein synthesis. So you don't need protein synthesis in order to make that because that happens--that is a mechanism that occurs on pre-existing latent transcription factors that all of a sudden turn on, as Bo Siesjo has shown, C-fos, for example.

DR. SIESJO: But, of course, you need protein synthesis for translation.

DR. VERMA: Right.

DR. SIESJO: And what may be happening is that through changes, whatever they are in this kind of single transduction cascade, you get a change in the priorities at the messenger RNA levels for protein synthesis. So this could be one of the initiation factors where this change in priorities occurs.

DR. VERMA: Is there much known about that topic, how to funnel certain messages through the protein synthetic apparatus versus others?

DR. SIESJO: I think for other tissues than the brain, but not for the brain yet.

COLONEL BELLAMY: I think we'll now break for lunch. We'll reconvene at about 1:30.

AFTERNOON SESSION

[1:33 p.m.]

COLONEL BELLAMY: Dr. Hochachka is going to speak, and he is going to tell usthat many animals have developed strategies for tolerating ischemia, hypoxia, anoxia, for a variable period of time.

DR. HOCHACHKA: Up until now we have been talking about animals that are super-sensitive to oxygen lack. What I'm going to tell you about is a species we have been studying that is extremely hypoxia- and anoxia-tolerant.

Some of you have heard the first attempt we made to try to understand how two systems work in this particular animal. I'll just give you a bit of a background on the biology of the animal. These are fresh-water turtles that live in North America, in Wisconsin, Minnesota, Ontario, Manitoba, and that's sort of part of the normal range of the species. They encounter extremely harsh winters. And to survive winter, like your animal, it goes into hibernation in a hibernaculum. The animal that we're studying goes into an over-wintering dive.

These are air-breathing animals, and what they do in order to avoid freezing, because they're ectothermic, they submerge themselves in mud, usually, in small lakes, ponds, or streams that are entering into--or leaving, I should say, from ponds or lakes. And they breath hold from about the middle of October until spring breakout, five or six months.

Many years ago, Ken Story and I published a paper in JBC in which we refer to them as facultative anaerobes, because under those conditions they do without oxygen for very long time periods. And then Don Jackson subsequently showed that at these low body temperatures they actually can survive six months in nitrogen. So he knows--I mean, it's experimented and shown that they can do what biologists knew they were doing. Just watch them.

Three students of mine recently addressed their attention to this problem, and this is just giving some reasons for looking at the animal. I don't think we have to repeat those right now--well, perhaps I will. First of all, that's obviously what I just said.

Their anoxic metabolic rate was known from measurements that Don Jackson made to be extremely low, and if you compared them to a similar size animal that was at 37 degrees, their metabolic rate, whole body or right off the tissue--you'll see this is true at the tissue level--was about less than 100, what you might anticipate.

Overall biological strategies of defense are known, and they look like they may be a good model for studying the reversible movement between near metabolic and normoxic metabolic rates, and this struck us as being an interesting possible model for the kind of goals that we have in mind with this group.

Three of my students--Les Buck, Steve Lynde, and Chris Dall--recently have made a study, and this is the strategy of their study. We took two different cell types. Two of my students, Buck and Lynde, were working on hepatocytes; Chris Dall was working on cortical neurons in collaboration with Peter Reiner in our neuro sciences group. So the small amount of neurophysiology I'll give you is from Peter Reiner's lab.

The plan was to quantify ATP turnover rates a variety of ways, to quantify ATP synthesis pathways, ATP demand pathways, to do exactly what Dr. Bellamy said would be useful, be able to create a proper energy budget, which I will show you we did; and, finally, evaluate the sensing and signal transduction systems.

I think perhaps when I outlined this problem to you previously, I probably started and just worked my way down this. Today what I want to do is start at this end, with Number 5, how does the animal or how at the cell level--we were looking purely at the isolated cell and tissue level. How does the system know when it's out of oxygen? Then we'll work our way back up the other scheme as to what the consequences of it are.

Well, of course, all of us know that the way organisms certainly at a biological level respond to oxygen availability depends upon the time course of the insult, short term, long term, or intermediate term. And the two kinds of responses that I'm going to look at I will refer to as instantaneous or immediate acute responses, or what I'll call intermediate responses, which actually in this case are 30 to 90 minutes long. Some people still call these early or short-term responses, but, in fact, they are very, very different. An acute response is very, very different from one that goes on in 30 to 60 or 90 minutes, and we'll show you what I mean by this.

My major interest in this actually started with trying to understand the role of oxygen in metabolic regulation, and we and others knew that there are many cells and tissues that behave as a function of oxygen concentration in their aerobic metabolism along this way; that is, the oxygen concentration drops, metabolism drops. You might say, fine, that's wonderful, you are just titrating cytochromoxidase. The problem is we're not titrating cytochromoxidase. These are data of Thurmond, et al., surviving in a--I actually took it from a book, "Surviving Hypoxia," but they obviously published this in the primary literature--whereas the apparent KM for this is very, very much above the apparent KM of cytochromoxidase. So if you looked at cells from mitochondria, that sort of curve is shifting enormously to the left.

When you have an integrated system, it became evident to several of us that the system was behaving as if it could sense oxygen some way, and that this in turn was signaling the cell to slow down aerobic metabolism.

Well, the way this is done in an acute system is not even known to this day, and we have some ideas on that, but I don't have time to really relate those to you today. Let's see. Is this going to work or not work? There it goes.

So what we did as a start is we took as a model of oxygen sensing the system that was worked out for erythropoietin biosynthesis, and as a result of work in several labs, of which the two that impressed me the most and are modeling our own thinking the most are Radcliffe at Oxford and Simenza working somewhere in these parts. Actually, I think he's at the University of Maryland.

At any rate, in the erythropoietin biosynthesis pathways, probably many of you know oxygen is sensed by a direct sensing mechanism which is most likely a heme protein. This turns on what Dr. Bazan referred to as a first round of gene activation, and signal transduction pathways there, unfortunately, that's the weakest part of this whole story, but it leads to the production of something called HIF-1, the first round of gene activation. HIF-1 is hypoxia-inducible factor 1. This is totally analogous to a growth factor, transcription factor, any of the kind of factors that Dr. Bazan listed in his summary graphs.

This is the transcription factor that actually is involved in regulating the EPO gene through two sites, a promoter site and an enhancer site, and leads to the production of EPO mRNA and then the production of erythropoietin.

Now, that part of the story probably many of you are familiar with. What I found particularly useful about the research that was done in this area was that over the many years of probing it, at least four experimental rationales were used; experimental means for probing this kind of an oxygen-sensing system were worked out. I list these here, and these are the probes that we used to test for this kind of system in our turtle hepatocytes.

The first thing is that molecular oxygen is absolutely involved in that. You simply cannot depress metabolism with cyanide and get the same response. So the signal for turning on HIF production, HIF-1 production, and, therefore, EPO production and activation of the EPO gene, involves a pathway that is sensing oxygen per se. Secondly, cobalt and nickel lock heme proteins in deoxy confirmations, and if it's a heme protein that is being used as the oxygen sensor, this should mimic anoxia.

Carbon monoxide, in contrast, locks heme proteins in oxy confirmations, and if we're titrating a heme protein, as we've moved down that oxygen concentration curve, then cobalt should reverse the anoxia effects.

Finally, if we are dealing with a heme protein, there are well-known inhibitors of heme synthesis that should also abrogate the effects of either cobalt, nickel, or anoxia. And these, of course, are in addition to what we

already know, that the system is responding to oxygen in concentrations that are well above that of mitochondrial metabolisms. So that in itself is kind of evidence for an oxygen-sensing system.

So what Steve Lynde and Les Buck did in our lab was to look at the expression of proteins under anoxic or extremely hypoxic conditions under these various experimental conditions, and the thing that I will be pointing out to you is cyanide anoxia, anoxia plus cyanide, and what you will see is the expression of some bands go up and some bands go down. And when he does this with nickel and cobalt, you see some bands going up, some bands going down.

If I summarize those in terms of tables, what we found in those studies were a series of four bands that we identified purely on the basis of their molecular weight, as characterized by standardized gel electrophoresis. And a question that was asked earlier on, even though there's a profound inhibition of protein biosynthesis under extremely hypoxic conditions, the expression of these proteins are all going up, and some of them by a very large percentage, by a very large magnitude.

Bear in mind that this is one protein out of all the proteins that in theory could be made, and so, in fact, the energetic demands of that are only--even under anoxic conditions, are a small fraction of what is actually being required.

The answer to your question is there is absolutely no doubt there is differential expression of genes, not only to the level of mRNA but into through translation, into the--

DR. BAZAN: How often do these change?

DR. HOCHACHKA: Thirty to 60 minutes. This is what I call--you referred to them as early response genes, and, in fact, we'll get back to that in another example.

In that same hepatocyte preparation, there were five proteins whose expression was inhibited, and the percent change is shown here. Some of them almost completely shut down. Others shut down less. So there was not just a generalized inhibition of proteins, though we actually have observed that, and I will relate what fraction of energy metabolism is due to that. But there was some preferential inhibition of these.

We thought these might be related to heat shock proteins, so we did a heat shock experiment and identified that the bands that were induced by heat shock were different from the ones we were dealing with. So we weren't dealing--they may very well be stressed proteins, or some of them may be stressed proteins, but they weren't stressed proteins that were equivalent to heat shock proteins.

On the basis of this--and you'll see some other evidence that came along--the kind of idea, the kind of model that we think is going on, are two cycles, at least two cycles of gene expression in response to oxygen-sensing, and

the evidence clearly indicates that it may be a heme protein. We have no idea who that heme protein is. Steve Lynde is still working at that. He's at Woods Hole at the moment, the MBL at Woods Hole, and trying to follow this up further. He's even getting more evidence that it's a heme protein.

We imagine there must be some specific binding site, regulatory site perhaps, that's involved in regulating minimally the expression of nine proteins that we're aware of.

DR. BAZAN: Have you considered the possibility that rather than gene expression, you have proteases activated and then your new proteins are fragments of other proteins?

DR. HOCHACHKA: At this stage we do. We can't rule that out, but we suspect that's not the case because of the inhibitor studies.

Also, at the same time we are doing this, Jungemann was working with rat hepatocytes, and what he found, he was looking at glucagon, regulation of gluconeogenesis. And he, almost by serendipity, discovered that that process itself was under oxygen regulation. And the system--in his case, he has a system that is under PKA regulation, involves the phosphorylation of signal proteins that bind at a specific site. He thinks that oxygen, through a heme protein, is involved in modulating this. So obviously the slide I just showed you was designed in such a way as to fit his data in with our data.

Now, in his case, I included a number of gluconeogenic enzymes that are known to be regulated by glucagon, but the one that he studied in detail was only PEPCK. And at the same time, glucokinase and pyruvate kinase are known to be upregulated under these conditions, as are other glycolytic enzymes.

So in the case of the turtle hepatocytes--the only part of the system we think we're dealing with is here--we have no idea what that pathway is. In the case of the rat hepatocytes, we think we're probably dealing with a homologous, if not--analogous if not a directly homologous system, which is also modulating glucagon regulation.

DR. HALLENBECK: You almost certainly said this, but I probably just missed it. What is your evidence that it's a heme protein?

DR. HOCHACHKA: Those four criteria I listed to you before.

DR. HALLENBECK: Yes, I just missed it.

DR. HOCHACHKA: Okay, the fact that the biosynthesis responded exactly to cobalt and nickel and heme synthesis exactly the way you would predict on the basis of this being a heme system.

We are not the only ones who have been looking at the effect of oxygen on gene expression at a cellular level,

as already Dr. Bazan has mentioned. Keith Webster at Stanford and Brian Murphy in Sutherland have been looking at this with isolate rat hepatocytes--I mean myocytes, neonatal myocytes. And the system that they found, that they have been probing is the cfos cjun system, which has already been mentioned.

In their case, they've got evidence for the signal--they have zero evidence for what the oxygen-sensing system is. So here there's the big question mark right there. But what they do have is a lot more information on the transduction of that signal into gene activation. They know that this involves a downregulation of the PKA activation pathway but an upregulation of the PKC activation system, the phosphorylate signal proteins involved in the regulation of cfos and cjun. Cfos and cjun were proteins that--you know, some of you here are very familiar with them. Most biologists don't know a thing about cfos and cjun, and that included me. When I read the literature, I was astonished by, A, how big it was and, B, how frustratingly disappointing it was. It's enormously detailed in terms of the regulation of this part of the system. And this is because cfos and cjun are oncogenes, and there's an enormous interest in it in cancer research.

What they do is where the problem begins to lie. What is the physiological significance of cfos and cjun, and my guess is that there are almost no papers on that. What is known is that they are inactive as individuals, as individual products; that they form heterodimers, which Keith Webster and others refer to as tertiary messengers, and that they're binding to a specific AP1 site on the DNA is itself oxygen sensitive, or at least redox sensitive. They think this may be simply because of a cysteine SH group on the jun.

Be that as it may, the evidence is very strong that a slug of genes whose expression is then regulated by the AP1 site is therefore regulated by the system. Included amongst those are glycolytic enzymes. Alpha actin itself is, metallothienin, and possibly heme oxygenase which is involved in something you'd be interested in, which is a scavenger system for free radicals.

Now, as I said, if it's glycolytic enzymes that are under the regulation of these, the function is obvious. But the point is most of this vast literature on cfos, cjun, says nothing concerning what the functional significance of these is. I have postulated that the net effect of these or maybe one component specifically of them is what is involved in downregulating the ATP turnover rate or the metabolic rate of these cells under hypoxia.

One final thing I wanted to emphasize is this: What will be obvious to you is, in all these summary diagrams that I've given you, there are large deficit areas, question mark areas where we're not really sure what the pathway is.

Peter Radcliffe and Chris Pugh at Oxford think that this system that started this whole--at least started my thinking off of this, which was the EPO system--in fact, by

serendipity, may have identified what they think will be common features to all of these oxygen-sensitive pathways we've just discussed. They believe that the oxygen-sensing pathway is universal, and the reason they believe it's universal is because this part of it, and especially the EPO enhancer sequence or this enhancer sequence, has been found in every cell line that they have searched for it in. And in some cell lines, the regulation of this enhancer is not involved in the regulation of EPO, but it's involved in the regulation of phosphoglycerate kinase and lactate dehydrogenase, for example, in the upregulation of these in glycolysis, the only tissues they have found so far where this enhancer works not for EPO but for other oxygen-sensitive genes. But the point they make is that this sequence and its sensitivity to HIF-1, hypoxia-inducible factor 1, is found in every cell line they have so far investigated, leading them to conclude that it, indeed, is a universal oxygen-sensing pathway.

Now, remember, I started off by describing to you one experiment in which oxygen concentration titrated metabolism down to zero. And then I present you a bunch of evidence on an oxygen-sensing pathway that takes 30 to 60 minutes to be expressed. Somewhere in there we believe that this pathway has an arrow that leads directly to the acute response in metabolism. We're not sure what that signal transduction pathway is or what components are involved in it. But I don't think that these pathways are separate from each other because the behavior, the acute response, cannot be interpreted sensibly except through an oxygen-sensing system. So even though in this talk I have no way of relating this kind of pathway, even if I accept that it is universal to the acute response, I actually believe that it probably is related to it.

If you take seriously this idea that these initial sensing systems are somehow involved in the metabolic response to hypoxia, what is the metabolic response of these cells to acute hypoxia? Well, at this point, I will definitely be getting into stuff that some of you have heard, so I will go through it very, very quickly. What we have found over and over again is in hypoxia-tolerant cells there is no attempt to make up for the oxygen lack, the energy deficit due to oxygen lack. They simply tolerate the lack of oxygen. And when you go down that oxygen curve, it is a true suppression of metabolism. The deficit in energy is not made up by activating anaerobic systems. As a result of that, if you measure it by heat output, you show this about a 90 percent drop in metabolic rate of hepatocytes. If you measure it--that's just a summary of those slides. If you measure it in isolated cortical neurons of turtles, you find exactly the same thing, this very profound suppression of metabolism. It's not as severe in the cortical cells as in the hepatocytes, but the direction is exactly the same.

Again, this is direct calorimetry, both in the previous slide and in this one. If you use metabolic criteria such as if it's a completely anoxic system, lactate

generation rate is a perfectly good measure of metabolism in these cells, or deoxyglucose uptake done in vivo, and we find again under anoxic conditions a very profound suppression of metabolism.

Chris Dall, while the hepatocyte work was going on, was doing simultaneous work using electrophysiological methods for studying the effect of anoxia on turtle cortical neurons. I'm not going to summarize any of his work except the metabolic depression part of it and also what's happening at the level of conductance of membranes, which already has come up as a possible large energy sink in these cells.

When we look at the ATP demand side of ATP turnover in cortical cells, we cannot give you a complete budget. But we know that there is a large saving that is occurring at the level of cell membranes which is based upon the inherent leakiness or the conductivity of those, which is measured electrically. At 37 degrees, the difference between the rat cortical neurons versus the turtle cortical neurons was four. What that means is that steady state, resting, normoxic conditions, the rat cortical neuron is putting out four times more energy to maintain the same electrochemical gradient across its membranes. Or if you like the membranes, four times more leaky. Or the conductance is four times greater than that.

When you do the experiment at 15 degrees, this becomes 23-fold, and you do it--when you make the comparison at 3 degrees--sorry, that should be 3 rather than 37. It turns out to be about 140. That's extrapolated from a temperature curve. There may be some error in that figure. So an enormous saving is being made by this animal at the level of the cell membrane.

Now, we've already realized from other kinds of information that that is an important site for us to concentrate on or to think about, and the nature is telling us exactly the same thing, that they save a lot of energy at that point.

Now, in the case of hepatocytes, when we look at the ATP demand and ATP supply system, we can give you a complete budget, fortunately, and I think that Steve Lynde and Les Buck deserve enormous credit for having managed to achieve that. I don't think you can go to the literature and find this kind of budget in more than perhaps one other cell. So we've done the budget under normoxic, under extreme hypoxic conditions, and let's just look at percentages here of the various ATP demanding functions. The overall ATP turnover, if we set that at 100, about 28 percent of that is Waughbain sensitive; 36 percent of that is due to protein biosynthesis.

Now, bear in mind this is hepatocytes, so these figures will be very different for different types of tissues. The liver is a protein synthesis machinery.

Protein degradation also costs energy, and it costs about 20 percent of the normoxic ATP turnover rate. Urea

synthesis, which is another job of this cell, is about 3 percent, and that, of course, could change and that might be different in a mammal than in a turtle. Glyconeogenesis under our conditions was about 16 percent. In fact, I think if you add all of those up, it's a bit more than 100 percent, but, you know, given the fact that they're done in different independent parallel experiments, we feel that we're pretty close.

What's fascinating is when you make the system hypoxic, you get about a 94 percent suppression of overall ATP turnover rate. Now, if we set that suppressed rate at 100 percent, the sodium potassium ATPase now accounts for three-quarters of the total ATP turnover. So of all the savings this cell can do, the poorest job is still at the level of the leakiness of the cell membrane. In order to maintain viability, in order to maintain electrochemical gradients, 75 percent of the ATP turnover that is still remaining in that cell is being used to achieve that end.

Protein biosynthesis, somebody who is curious about that, it drops down to about 25 percent of the remaining, so that these two functions account literally for almost everything that is there. Here you see our addition is even a bit further off 100 percent because we estimate that about 12 percent is due to protein degradation and urea synthesis is even a wee bit less. But there's no doubt that protein turnover and sodium potassium ATPase account for the bulk of ATP turnover rate under these conditions. And of those two, this is by far the more important one.

You may want to ponder that, because that is the sort of thing that Dr. Bellamy would like to know for other organs and tissues.

Okay. Those measurements of ours suggest that ATP utilizing pathways and ATP production pathways are both downregulated. And if they are downregulated in a coordinated way, in step, in other words, you would expect no perturbation in the concentrations of ATP or the end products of ATP in steady state. This, in fact, is observed, and I'm not going to dwell too much on this. This happens to be for hepatocytes showing that these changes don't occur. These are data of Peter Lutz and the group in Miami on the anoxic turtle brain in vivo, and again, within experimental error, the ATP concentrations are maintained for up to two hours of--in this case, it was just nitrogen breathing--where anoxia of the brain was determined by reading the redox state of cytochrome aa3. So it was a complicated physiological experiment showing about the same thing.

In this brain, the only two metabolites that have been measured that are changing are those involved in anaerobic metabolism, the degradation of PCR and the accumulation of lactate from endogenous licogen or plasma glucose.

So then in summary--

DR. SIESJO: Is the decrease in phospo- (?) due to a rise in the pre-ATP concentration, do you think, or is it due to the acidosis?

DR. HOCHACHKA: I don't know, honestly. I have been studying crato-phosphokinase, as you just mentioned, in vivo and it has never occurred to me what causes the shift--it's an enzyme that stays very close to equilibrium. So I'd probably say that any of the substrate products that are changing, even slightly at the active site, would cause the change in flux. So the usual interpretation might be that local concentrations of ATP are actually dropping slightly. ADP would, therefore, rise slightly, and that may be the initial driving event for shifting the equilibrium, you know, bringing it back into equilibrium. But that's not very well understood, and it could be hydrogen ion, as you suggest.

DR. SIESJO: Because you are going up to about 20 in lactate.

DR. HOCHACHKA: That's right.

DR. SIESJO: That could be acidosis.

DR. HOCHACHKA: Yes, that's right. I mean, these are--what we find--these curves look fairly symmetrical, and in one preparation, a highly glycolytic fast switch fiber that we studied, one of my students studied, he found that the clearance of lactate or the production of lactate and the degradation of PCR or the resynthesis of PCR, they were bang on, one to the other. And that linkage we thought was being driven by hydrogen ion. So it probably is in some systems that they are post--but mainly in mitochondrial-rich muscles, that correlation breaks down. So it's not quite as--it's got to be driven there by the adenylates I would think, but I'm not sure.

DR. SIESJO: If you build up hydrogen ions, and if you increase acidosis, you would aid a lot of metabolic reactions, and you would block a lot of the cation channels in the membranes.

DR. HOCHACHKA: Yes. That's a very good point. I don't have an answer to that except to say it should be--it has got to be looked at in these animals. It's something that needs clarification.

COLONEL BELLAMY: Would you rephrase that, the intracellular acidosis would have the effect of blocking cation channels?

DR. SIESJO: Yes.

COLONEL BELLAMY: Did you say that?

DR. SIESJO: Yes. It was previously known from muscle tissue that voltage-sensitive calcium channels were sensitive to changes in the pH, and particularly from the outside of the channel, so you reduce conductance of these channels with acidosis. Now it has been clearly shown that with acidosis you will block currents through NMDA-gated calcium channels. Therefore, you will reduce calcium flux

through the NMDA gate. But we checked it in vivo with ischemia and the flux is there. Then it was found that not only lactic acidosis but also CO2 could--

DR. HOCHACHKA: Just an artificial increase in ion concentration.

DR. BENTLEY: Those tissues--

DR. HOCHACHKA: Well, there are lots of people--sorry, go ahead.

DR. BENTLEY: Those tissue there weren't particularly acidotic because those turtles were breathing nitrogen and they were blowing off their CO2.

DR. SIESJO: Yes, but you have lactate after--

DR. BENTLEY: Still, they had a respiratory compensation for that metabolic acidosis.

DR. SIESJO: No, you can't compensate for 20 unless you get rid of the hydrogen ions from the system. Because the CO2 compensation for 20 would require an enormous decrease in PCO2.

DR. BENTLEY: They do undergo a pretty big decrease in CO2.

DR. HOCHACHKA: I don't know what was happening to intracellular pH in these, you know, but the turtles are incredibly well buffered in the extracellular fluid. And even after months and 200 millimolar concentrations of lactate, 0.2 molar, the pH is only 6.9.

DR. SIESJO: Okay.

DR. HOCHACHKA: But intracellular meaning in this case, you know, it could very well be--I think your point is extremely well made, and, you know, it's just up in the air. The answer is not in. But it's an important theoretical possibility.

Very briefly, in summary, what we think is going on is this: Short-term response, we don't know the nature of oxygen sensing. We think there's some indirect evidence for it. In the intermediate response, there's no doubt some clear-cut evidence for a direct effect of an oxygen-sensing pathway upon the preferential up- or downregulation of specific components.

Now, we're guessing at what these are in the case of turtles. In the case of rats, we know some of them in the hepatocytes, and we know others in the case of myocytes, and obviously the cfos, cjun system is seen not only in myocytes but other tissues as well.

We suppose that related with this or with the initial sensing is this general downregulation of ATP turnover rates so that the animal is saving energy; and if it is saving carbon and energy, it's doing this not only in order to save energy but to avoid poisoning itself with anaerobic end products. A big saving we think is occurring by adaptations occurring at the level of cell membrane. But

despite those adaptations under complete anoxic conditions, this still is the primary sink for ATP turnover, for the ATP demand side of the ATP turnover cycle.

This just happened to be in my slides, so I didn't take it out. If I ever talk to general biologists, I have to explain to them why it's advantageous and what are some of the consequences of metabolic suppression under these kinds of stressful conditions. This is the Rip Van Winkle effect we're all interested in. This is something that occurred to me when other people were talking here, especially in the hibernating field, where you get this hypometabolic state for at least a week-long period, week-long to perhaps two-week-long periods, when some proteins have half-lives of only hours. And yet protein biosynthesis is at a level of 1 percent of normal. I think the answer to that is that there are unknown mechanisms for improving the stability of many macromolecules, and in some invertebrate systems there's extremely good evidence that, for example, cytochrome oxidase, normoxic turnover rate of 25 to 30 hours, anoxic metabolically suppressed to an extreme degree, the half-life of that becomes almost immeasurable, 77 days. That's a very crude estimate. So there are some very, very important effects occurring there that we know nothing about at this point.

Okay. I could stop there. If I'd say anything, it is just to remind you that some of these things could work with real animals, i.e., in mammal, and I would give you three or four slides on the seal. How are we set for time, Dr. Bellamy?

COLONEL BELLAMY: You have 10 minutes.

DR. HOCHACHKA: Okay. Well, I'll just very quickly describe to you this animal.

Our lab has been working on hypoxic defense mechanisms looking at different kinds of models. This has been one of our best models. It's the Antarctic Weddell seal, and the way we study it is to study them in sea ice where we drill a hole through the ice, capture a seal, instrument the seal just like the telecommunication that Dr. Bellamy was talking about in the first case. We use microprocessors, small computers, about equivalent to the old Apple IIc's, that sort of power, far more than what our brains can actually create in terms of useful measurements that we can make on them. Then we release the animal, so it goes off to swim whenever it wants for whatever time period it wants, to whatever depth.

Now, they always have to come back because it's the only breathing hole within about a 5-kilometer radius of where we've set up. So when they return to the breathing hole, we have a float. Usually the backpack is carried in about this position and about a meter-long float comes up to the surface, and we communicate with the onboard computer. We transfer the information by fiber optics, so it takes about a picosecond to transfer a day's data.

We know that these animals are extremely good at harnessing and managing oxygen down to what for a mammal are ridiculously low concentrations. Last year we went down there, and we put a myoglobin meter right on the--attached to the fascia but right on the latissimus dorsi, dorsal lat, latissimus muscle, and monitored the myoglobin saturation in the muscle as a function of time of diving.

What we found was absolutely fascinating. This is a dive of about half an hour duration. We had some dives that are short, about 5-, 6-, 10-minute duration, which to this animal is almost nothing, and we had some even longer dives, about 40 minutes. We never did manage to get one of their record dives because they just didn't feel like giving it to us when we're monitoring information.

The point that is interesting is they would titrate down that oxygen precisely to the same level every dive. So they knew exactly what they were doing. It was an explicitly controlled system. At the end of the dive, they'd obviously bounce back as they began to re-oxygen load the system. They would pop it right back.

COLONEL BELLAMY: The ordinate has velocity meters per second.

DR. HOCHACHKA: This is the velocity. Velocity is zero when he's sitting at the hole. He jumps up in this case to one meter and then bounce around, about 1.5 meters a second. They always swim between 1 and 1.5 meters a second. So people who work in this field are tired of putting velocity meters on them because they always swim at the same speed.

COLONEL BELLAMY: The lower line is saturation of muscle--

DR. HOCHACHKA: This is desaturation of myoglobin in this direction.

COLONEL BELLAMY: I see. Okay.

DR. HOCHACHKA: Now, let me remind you of something else. If this were a closed system and it was just myoglobin in a test tube, what would happen is this thing would come down towards an asymptote, and experimentally we know that there's an asymptote at about 10 knots in a totally ischemic muscle.

COLONEL BELLAMY: Myoglobin is usually 100 percent--close to 100 percent saturated?

DR. HOCHACHKA: Pardon me?

COLONEL BELLAMY: Myoglobin is usually very--

DR. HOCHACHKA: Oh, yes. Yes. Here it gets close to desaturation but never is actually run right down to zero. So they go to extremely hypoxic--I think what they're doing, the tissues that are not working hard, at least those tissues we know anything about, I think are going down in oxygen saturation here. Just like that first slide I showed you on the acute response.

Yes?

DR. SAFAR: Arterial PO2 stays normal?

DR. HOCHACHKA: Arterial PO2 drops down. Now, bear in mind that the lungs stop serving as a gas exchange organ.

DR. SAFAR: Total flow is concentrated to how much?

DR. HOCHACHKA: What do you mean total flow?

DR. SAFAR: In the diving seal, vessels close off, except to the heart and brain.

DR. HOCHACHKA: Okay. Yes, you're referring to the physiological defenses the animal turns on. I forgot to mention those. I was going straight into the biochemistry.

When the animal breath-hold dives, especially in long dives, what they do is they activate a severe bradycardia with peripheral vasoconstriction. The lung is shut off at about 30 meters depth, so the lung is not a gas exchange organ. It's just a tissue. That actually is important in metabolical homeostasis, but it's not important in gas and CO2 homeostasis. So as a result of that, the PaO2 is actually dropping to quite low levels.

DR. SAFAR: But slowly.

DR. HOCHACHKA: Slowly, yes.

DR. SAFAR: Because it's only here and there.

DR. HOCHACHKA: Yes, it's there and in other places, but at the lower rate in other places. See, what this says is the circulation here is being--there's a lower rate of delivery of oxygen to it. And if it weren't, this would asymptote out. The main reason I knew that this was physiologically controlled was that there's no sign of the shape of a curve that you get for isolated myoglobin, having the oxygen pulled off it. That was the first evidence. Then the second, of course, is we just calibrate it. We know when this gets down to zero.

DR. SAFAR: It's measured where?

DR. HOCHACHKA: Myoglobin is measured in the animal's muscle right at about this position.

DR. SAFAR: Which is active?

DR. HOCHACHKA: Yes. Active like in marathon runners, but it is active.

DR. SAFAR: Almost not perfused.

DR. HOCHACHKA: Perfused at a low rate, and according to what it can do. It's probably why he only swims at one meter a second, even though he's three meters long.

Okay. These animals can do this for long time periods. This is the Southern elephant seal rather than the Weddell seal. They're similar size, and as far as we know from physiology and biochemistry and diving behavior, there's no reason why this record should not be attainable by any one

of these species. This happens to be a 2-hour dive right there for a 300 kg animal.

The fact that we know they go hypometabolic is if you calculate the resting metabolic rate, they consume all onboard supplies of oxygen at resting metabolic rates within 30 minutes. And since they dive for 2 hours and don't accumulate toxic quantities of anaerobic enterides, we know they are hypometabolic. Their metabolic rate when they're at sea is probably resting levels or lower, substantially lower. We think it's substantially lower, about perhaps half what it normally is.

The fact that they can do this frequently is shown by this slide. This is a time-depth record for an individual animal that was monitored for a couple of days. Here is one day's dive over 12 hours, during which time that animal only took eight dives. So every dive was over an hour long.

Mike Fedack at Cambridge has tracked these animals over very long distances. This is an oceanographer's sort of three dimensional version of an underwater sea mountain, and these are the tracks of a Southern elephant seal as she foraged for periods of about six or seven weeks in this particular case. You can see what she did. She loved the edge of this mountain, and she spent a lot of time feeding along it. So this is just a way of life for these animals. What they're doing is that every time they go down, they're suppressing down to at least a third or half normal.

This is my concluding slide. The main features of hypoxia tolerance include: a heme protein-based oxygen-sensing and signal transduction pathway that tells the cell when to initiate defense. That's occurring at the cell level. It includes a set of genes, example for glycolytic enzymes. We know that must be true at least in some tissues whose expression is upregulated in hypoxia. We think that maybe four of the bands that we saw go up in these cells, and at least one or more of them may have been glycolytic enzymes. We don't have any proof of that at this stage.

We had acetogenes, example for glyconeogenesis based on the hepatocyte work of Jungemann for PEPCK whose expression is downregulated in hypoxia.

Brian Murphy and Keith Webster have shown the Krebs cycle enzymes are downregulated under these conditions, so we think that probably is the same system but we don't know that for sure.

We know a generalized decline in ATP demand pathways occur in hypoxia. For at least one cell type, we can fully quantify this. For another cell type, we cannot, but we can certainly say that nature has done a lot of adapting at the level of the cell membrane.

There's a generalized decline--oh, here, I'm just restating that--a generalized decline in membrane permeability. We have flippantly referred to this as channel arrest, and Szick and Rosenthal at Miami in the turtle brain have referred to this as spike arrest. There is an enormous

suppression of electrical activity of the brain in the turtle under these anoxic conditions.

By the way, the way that is achieved, there is an increase in the concentration of inhibitory amino acids, and I mentioned to you in private conversations the suppression of glutamate production. So glutamate concentrations, the excitotoxicity of glutamate is avoided. The other thing I mentioned is within the first 300 minutes, longer in what our experiments were, Peter Lutz and Goren Nielsen, using microdialysis techniques, have shown a large pulse of adenosine that comes in. They believe it plays a very, very protective role during the first perhaps couple of hours or so.

COLONEL BELLAMY: And that's the breakdown of ATP, I suppose?

DR. HOCHACHKA: They think it comes from ATP, but, of course, you must realize the amount of adenosine that you need for the signaling functions is a very minute fraction of the amount of ATP that is there. So the influence on the ATP pool is actually fairly trivial. But they certainly believe that that is the source of it.

DR. BAZAN: But is it in the (?) pool of ATP, adenosine?

DR. HOCHACHKA: Nothing is known about that in the turtle brain, but I guess that's possible.

DR. BAZAN: No, in mammalian brain.

DR. HOCHACHKA: That I did not realize.

DR. BAZAN: Yes.

DR. HOCHACHKA: This could very well be the case then.

DR. SIESJO: They would get it from ANP, wouldn't they?

DR. BAZAN: Yes.

DR. HOCHACHKA: Yes, well, that actually comes from ANP, you're right. Finally, and most importantly, there's a maintained balance between ATP demand and ATP supply pathways so that the adenylates remain in normal range.

Now, this, I was reminded by something you said. When I came to this concluding point, there is something I should emphasize; that is, in any hypoxia-tolerant cells that we've seen, whether they be neuronal cells, heart cells, muscle cells, or liver cells, most of the data being on liver and brain--from our lab, at any rate--it is true that hypoxia-tolerant organisms essentially always defend ATP concentrations when they run into these extreme oxygen limitation conditions. So even though the metabolism is suppressed and oxygen is not available and all that sort of stuff, they do protect that. People, as a result, have been tempted to say that that is, indeed, one of the most

important things you need in order to defend a cell against hypoxia or anoxia.

You pointed out in your examples--of course, these are hypoxia-sensitive nervous systems you're looking at--that very early on ATP concentrations are drastically depleted. However, in studies like that with mammals, as I'm sure you realize, it has been shown that the ATP depletion is not the cause of the demise of the cell. It's impossible--and it turns out in the case of a couple of invertebrate systems, they don't bother to defend the adenylates. Any of the ones we've ever looked at, they always do defend, but it may not be necessary that they do. And certainly in the case even when they don't, it's not ATP depletion per se that causes the cell to die.

I think the best evidence came from the heart where they could show perfectly good recovery in hearts even though--

COLONEL BELLAMY: Is there a slide here?

DR. HOCHACHKA: Yes, that's all. That's it.

MAJOR BRUTTIG: When you're talking about membrane-mediated events and you think about an animal that lives at 37 degrees totally, the membrane being made up of so much lipid and such small amounts of protein and the protein getting tertiary, quartiary structure changes within that lipid membrane, okay--

DR. HOCHACHKA: Right.

MAJOR BRUTTIG: Now when you change temperature, you would expect to be approaching the point at which you'd get a phase change in the membrane and a change the speed with which these proteins are going to deform in that membrane. Do you know anything, number one, about the lipid concentration differences between, say, the turtle and a typical mammal to include maybe the seal? And as the turtle approaches cold weather, do those lipid changes alter the distribution?

DR. HOCHACHKA: There are several questions there you incorporated into one. That area is very, very extensively researched, and, yes, what you say is totally true, that the fatty acid composition of the phospholipids that make up the membranes, you know, are adjusted as a function of temperature; the fluidity in these animals is maintained right past zero. But in the case of mammals, if you measured a lipid-dependent function, you'd find a phase change at about 21 or 23 degrees Centigrade. In hibernators that doesn't occur, because they either defend against it before they go into hibernation, or their membranes are always fluid down to a much lower temperature. So that is an important consideration.

In the case of the turtles, however, we cannot account for these conductivity changes by a possible lipid phase change because they are in a fluid state to temperatures down to the 3-degree Centigrade range. On the

other hand, when many rate functions, especially metabolic rate functions, have been measured in turtles and in whole animal and in some isolated tissue preparations at around 11 degrees, 10 degrees Centigrade, there's a change in the areneous plot and much higher Q10s. So biologists interpret this as the animal using temperature as a means for sliding down; in other words, temperature being used aggressively to off-switch metabolism; and in the springtime, the reverse. When things begin to heat up, they can very quickly activate.

So the Q10 over the 0 to 10-degree range is very high. However, when we looked at that in isolated liver cells, there was not a sign of it, and we never really properly addressed that in the central nervous system, in the cortical cells, so we're not sure exactly what happened there.

That's quite an interesting point because people have asked, What's the basis for those conductivity changes? And I believe it's temperature-dependent modulation of channels, because I don't think there is any evidence that phospholipids are changing phase during that time period.

DR. SIESJO: This is a fascinating story, and I really love it. Is there any information on whether the effect on conductance is exerted on sodium channels or on all types of cation channels?

DR. HOCHACHKA: You're getting me there into an area that I want to tread in very carefully, because it's not really my turf. But Peter Reiner did not believe that these were sodium channels that were being modulated in the turtle cortical neurons. He thought they were--and I hope I am paraphrasing him correctly--generalized leak channels. I think it was potassium leak channels he was mainly thinking of.

DR. SIESJO: Of course, the leading event would be sodium influx when you have deliberalization --

DR. HOCHACHKA: Yes.

DR. SIESJO: My second question would be: What is known about the calcium fluxes across membranes here? Could you conceive of a mechanism like change of phosphorylation which would change the conductance of cation channels?

DR. HOCHACHKA: Yes, I'll tell you what is experimentally known, only if you promise not to tell anybody. Steve Lynde phoned me and told me this in a state very high excitement. He's at Woods Hole. He used what's called a vibrating probe, a microprobe, for monitoring oxygen concentrations down to picometers from the cell surface. So they can tell you the concentration of oxygen essentially that the cell has seen. They're working with isolated turtle hepatocytes, just as he was in my lab.

What they then did is they loaded up the cells with calcium-45, and they did the reverse of what you presented in your slides. Instead of calcium uptake, what they measured with calcium--the oxygen dependence of calcium eflux. It's a

wonderful result. There's enormous oxygen sensitivity with a KM that's well above 10 micromolar, way above the KM for mitochondrial metabolism.

Basically, by 10 micromolar concentrations, calcium eflux has fallen to anoxic levels. As far as that cell is concerned for calcium regulation, at least for that flux of calcium, the cell has said we are out of oxygen, which is an amazing result, really fascinating.

DR. SIESJO: That is fascinating, but nothing is known about the influx of calcium.

DR. HOCHACHKA: At this stage, nothing. I mean, I should probe Steve as to why they started with eflux, but I think it was the way they were doing the experiment. It was easier for them to measure it in that direction.

DR. SIESJO: Yes, because one good way of closing down the mitochondrial metabolism would, of course, be to decrease the free cytosolic calcium concentration.

DR. HOCHACHKA: Yes.

DR. SIESJO: That you can either do by revving up the eflux or by hindering the influx.

DR. HOCHACHKA: Yes, yes. We just don't know about what the intracellular concentration is. Phil Bickler is working with Les Buck in San Francisco. They are interested in trying to run that down. But they didn't have the data the last time I saw them. That's still being probed.

COLONEL BELLAMY: Thank you, Dr. Hochachka.

Dr. Verma, who recently graduated from the neurology program at Walter Reed and is presently assigned to Uniformed Services University, will give us a brief presentation on ion channels.

DR. VERMA: Good afternoon. I want to take the opportunity to thank Dr. Bellamy for inviting me to share what I can in 15 minutes about a very large topic. I'm going to talk about ion channel function in hypoxia and ischemia and duscuss some ways that ion channel function can be directly modulated by metabolism, and by intermediates of metabolism. I have one slide, and the rest I have as transparencies.

Could I please have the first slide up?

This slide basically shows you the anatomy of an ion channel. We've come a long way since Hodgkins, Huxley, and Kats, and the marriage between protein chemists, molecular biologists and electrophysiologists has yielded a lot of information on what a channel does and what the channel is.

Typically, we think of channels as sort of doughnut structures that span the membrane. Most ion channels are made up of several sub-units. They're typically heteromeric, being composed of one of several sub-units in different combinations. So far several families of ion channels have

been identified, some that are voltage-sensitive, other that are ligand-sensitive. Ligand-sensitive channels share particular features in that they usually have about five sub-units that assemble to make a heteromer through a membrane. They have a membrane-spanning portion and a large extracellular portion, with a potential recognition site for a modulatory ligand. They have a central pore lined by hydrophilic amino acids that allows the channel to gate ions through, and they have a gating portion which determines the ion selectivity.

Some of the loops of these sub-units also have potential regulation sites on the inside of the membrane which may involve binding of intracellular ligands or binding of cytosolic and cytoskeletal proteins, perhaps interaction by G-proteins found in the membranes, and also sites for protein phosphorylation which figure very prominently in the regulation of channel function.

So far two main families of ligand-gated channels have been elucidated. One family includes the nicotinic acetylcholine receptor, the GABA A receptor, the serotonic HT3 receptor, and the glycine receptorEven though some of these channels conduct chloride or anions and some of them conduct cations, their subunits are actually very similar if you look at their amino acid composition. Another family is composed of the glutamate receptor channel sub-units, including the NMDA type and the non-NMDA type.

There are many levels of regulation for ion channel function. That's one of the main messages I want to leave you with today.

First of all, one can regulate ion channels directly at the level of the protein. This is an immediate effect that can take place without any gene transcription or protein synthesis, and several such mechanisms are known. We're most familiar with the ligand-gated aspect of channel regulation by, for example, glutamate, GABA, and acetylcholine. Many of the toxins that might be generated in a disease process, whether it's ischemia or hypoxia, can also act directly at the level of the channel protein.

A variety of second messengers are also known to regulate channel function. Many channels are sensitive to direct activation by calcium, for example. Several potassium channels are directly sensitive to changes in systolic calcium concentrations. Sometimes calcium regulates these channels by itself. At other times it requires the cooperation of a calcium-binding protein such as calmodulin to do the job. Cyclic nucleotides also can either directly regulate channel function, again, by allosteric interaction with the protein, or they can secondarily affect channel function by activating their specific protein kinases.

In the last several years, there have been a number of channels identified that are turned on by G-proteins, which are subsequently turned on by another receptor mechanism. So the ion channels can actually serve as a common endpoint for many changes that occur in signal

transduction mechanisms. So in this scenario, changes that might be seen in ion channel function in response to ischemia or hypoxia would actually reflect the changes taking place in several signal transduction mechanisms with the channels being sort of the final endpoint, or one of the endpoints of that process.

By far the greatest amount of information we have as far as ion channel regulation is through protein phosphorylation. There are numerous examples of phosphorylation of ion channels, and all the kinases that have been studied so far--the cyclic AMP dependent (PKA), cyclic GMP dependent, calcium calmodulin dependent kinases, tyrosine kinases--can all use channels as substrates for phosphorylation. Phosphatases are equally as important in regulating protein function as kinases, and many of these phosphatases are coming to be known only now. We haven't really appreciated them very much and haven't had many specific probes for them. Phosphatases complicate the picture even more and allow a lot more diversity in the way that phosphorylation can regulate an ion function.

There are several examples of regulation of channel function by phosphorylation, and many neurotransmitter receptors have shown this phenomenon. The nicotinic acetylcholine receptor, for example, changes its rate of receptor desensitization upon phosphorylation while the GABA receptor, when phosphorylated can change both the magnitude and the kinetics of the chloride fluxes through that channel.

For the glutamate receptors, there has been a lot of work on both the non-NMDA and the NMDA receptors, PKA, for example, has been shown to increase currents through the non-NMDA receptors while phosphorylation of one of the sub-units of the NMDA receptors has been shown to modulate the magnesium sensitivity of that channel. A lot of regulation that can thus go on via second messengers acting directly at the membrane level to acutely affect the overall ionic function of that particular membrane.

Voltage-dependent channels are also extensively phosphorylated, and L-type calcium channels as well as certain potassium channels are prominently affected by phosphorylation in terms of both the amplitude and the speed of the conductance.

Aside from second messengers and protein phosphorylation, in the last several years we've also appreciated some other mechanisms that regulate channel function that are probably very important in ischemia and hypoxia--physical forces, for example. Channels have been identified that are directly sensitive to stretch and various tensions placed on the cell. Such channels may play a large role in clinical phenomena such as edema or deformation of tissue from trauma. We really don't have any pharmacological tools that address those channels yet, but such a target is conceivable. This may be an area that is worth looking into further in terms of treating victims of trauma.

Ion channel function can also be directly affected by various metabolites that might be altered in ischemia and hypoxia. There has been numerous demonstrations that ATP levels can directly affect ion channel function independent of phosphorylation. Most of this evidence has come from using ATP analogues that are unable to be hydrolyzed and also by direct binding studies. One of the most prominently studied channels in this regard is the ATP-sensitive potassium channel.

This channel is actually found to be closed by physiological levels of ATP. So when ATP levels drop, this channel opens and it has a potassium conductance that shows some slight rectification or directional preference for the current. In general, this channel tends to hyperpolarize cells, thereby decreasing their ability to fire. This has many profound effects in several organ systems.

In cardiac cells, for example, opening of this potassium channel markedly shortens the duration of the action potential. It also affects insulin secretion by the beta cells in the pancreas and has been shown to decrease neuronal firing. In many instances, opening of this channel can show protective effect in models where ischemia and hypoxia are being studied.

Several drugs that can mimic or antaginize the effect of ATP on this channel. For example, the sulfonylureas can close that channel much like elevated ATP levels do. There are some newly discovered drugs that may open this channel. Agents such as Nicorandil, Pinacidil, Chromakalim, and Diazoxide may soon offer therapeutic opportunities by allowing hyperpolarization of excitable cells during hypoxia and ischemia. So, again, even though this channel is a metabolite sensor, we may actually be able to pharmacologically mimic or make the channel think that the metabolites are at a certain level when they really aren't.

COLONEL BELLAMY: I'm sorry. Sodium is conducted through these channels?

DR. VERMA: Actually, these are predominantly potassium channels. Very recently there have been reports of some ATP sensitive sodium channels. While initially it was thought there was only one particular channel like this, it is now appreciated there are probably different isoforms of that channel that may have differing Km values for ATP and that may be found in different cell types. There has even been one channel, for example, that has been found in the mitochondrial inner membrane that is a potassium channel, but it is directly sensitive to ATP levels.

In addition to ATP, many channels are also affected by the complex of magnesium and ATP. Both of these molecules are very high in the cell, so in many conditions, they actually exist as a magnesium ATP complex. What is interesting is that ATP and magnesium-ATP sometimes show different effects on a particular channel.

For example, if ATP will activate a channel or close a channel, in some scenarios the Mg-ATP complex won't have any function.

In addition, magnesium, which is a very small ion is able to plug up a number of channels. Magnesium has been shown to inhibit a variety of channels.

As ATP levels rise and fall in a cell, the ratio of the magnesium-to-magnesium-ATP complex changes. And it may be that the channels are directly sensitive to such changes, so that if ATP levels drop, you have less of this complex and more free magnesium. Whereas, if ATP levels rise, you have more of this complex than you do of free magnesium. Just a simple buffering system for ATP and this cation with channels may serve as sort of a metabolic sensor. And many channels in that regard might be thought of as general energy sensors. Some of these mechanisms for regulating membrane function very rapidly may actually be built in directly into the channels. How many channels there are like that? Where are they? You know, how can we manipulate them? I think those are all very excellent questions to focus on in this regard.

What is extremely exciting is the recent demonstration of regulation of ion channels by oxygen directly. Now, oxygen--and this observation to me is very exciting. It was first made in the glomal cells of the carotid body, and what was demonstrated was that there was a direct effect on potassium currents by the level of oxygen. These cells are known to respond very rapidly to changes in oxygen tensions by changing their firing frequency. And using patch clamp studies where the investigators were pretty sure they had only like one or two proteins in their patch, they could demonstrate a direct effect of oxygen tensions on the potassium current in these isolated patches.

As the oxygen tension dropped, the potassium current dropped, and the net effect was that as oxygen tension dropped, these cells tended to depolarize and then that was proposed to directly imply a mechanism whereby oxygen could change the firing rates of these cells.

What has been equally exciting is that these channels have since been found in other cells. They haven't been found in all cells, but they've been found in certain unique places, for example, in the pulmonary vasculature myocytes. They have also been found in the neural epithelial bodies in some of the airway passages in the lung. They've also been found in some septal neurons, as well as some other neuronal ganglia.

So where these channels are and what functions they serve is not really clear yet, but it does seem to be clear that within a patch that contains only a couple of proteins, maybe just one protein, you can demonstrate a direct oxygen sensitivity of an ion current.

Now, what the sensing mechanism is of this is unclear. Clearly, we're removed from the whole transcription by hypoxia-inducible factor and so on because we have a

single membrane patch. Whether the sensor for oxygen is directly on the channel or whether it involves some other proteins that are associated with it in the channel isn't clear.

There has been some proposals and some sort of weak evidence to suggest that there may be other proteins involved. Some scenarios that one might envision is that there may be a protein that binds oxygen is making some metabolite based on the level of oxygen. For example, a protein like NMDA pH oxidase that is continuously making hydrogen peroxide, its product would be directly sensitive to the level of oxygen. And as oxygen levels dropped, perhaps that product is no longer made, and that product may have some activating function on the channel which, upon being removed, closes the channel. Again, most of the evidence for this involves sort of dirty inhibitors and is not that strong.

Alternatively, there may be another protein in these patches that senses the oxygen, but then binds to the channel and autoserically regulates it. Again, there's a lot of questions here, but, still, it's very exciting that you can demonstrate an effect of oxygen at such a small level.

Well, all of this, again, in a nutshell, there's a lot of information here, but all of this regulation by metabolites and by oxygen can potentially happen at the protein level. But ion channel function should not just be thought of at the protein level, and I think especially in this topic of hypoxia/ischemic, we need to think more about the role of regulating channels in a broader picture, in the broader scope of cell biology.

Ion channels can be modulated in many ways to subserve cell function. One can sort of aggregate ion channels in a particular part of the cell and polarize that cell to do a particular function. Ion channels can be used to perform metabolic compartmentation. For example, in muscles cells, in neuronal cells, there are ER compartments that are hooked up with calcium channels found in the plasma membrane. The phenomenon of calcium-induced calcium release, for example, that mediates skeletal and cardiac muscle contraction involves such a compartmentation and interaction between channels.

There are many examples of metabolic machinery being partitioned onto channels. For example, many glycolytic enzymes attach directly to channels, not only on the plasma membrane but also in the ER and also in the mitochondria.

The voltage-dependent anion channel of the mitochondria is a docking site for hexokinase and glucokinase, and it has been thought that that creates a local metabolic environment where fresh ATP can come out and stimulate the kinase and the ADP produced by the kinase goes back into the mitochondria, and you sort of short-circuit the system for a particular purpose.

There are also very important ion channels in organelles that we know very little about right now. One of them was mentioned earlier with respect to the cyclosporin sensitivity of a calcium release channel that has been found in the inner mitochondrial membrane.

Most of these changes that are happening in terms of compartmentation and so on are, again, probably mediated by post-translational covalent modification of these channels by phosphorylating these channels, affecting them with some second messenger and so on. But we can also regulate channels by using the fresh synthesis of channel protein, and this can happen by either changing sub-units of channels and sort of differentiating them. I mentioned that channels are usually heteromers of many different types of sub-units. It turns out that you can actually make zebra channels by putting in whatever sub-unit combinations you want, and those channels end up having different conductances and different properties, and potentially different levels of regulatability. Obviously, you can also regulate the number of channels, either in up- or downregulation.

Most of these effects involve not only RNA synthesis but protein synthesis, and how these might be regulated by hypoxia and ischemia has been alluded to by some of the previous speakers, and I just wanted to go over a little bit of this because we're interested in this topic and are doing some work in my lab on this topic.

Oxygen can actually regulate the synthesis of a variety of proteins in many cells. A great example of this has been recently elucidated in facultative anaerobic bacteria, where it has been found that these cells turn on nitrogen fixation genes in response to hypoxia. Using mutational studies, there's actually been an oxygen receptor that has been identified in these cells. The protein turns out to be a hemoprotein kinase. Half the protein is a heme protein, binds oxygen; the other half is a kinase. The level of saturation of the heme group actually regulates the kinase activity.

The protein, when oxygen levels drop, actually autophosphorylates itself and then phosphorylates the transcription factor, which then turns on transcription from the chromosome.

Similarly, oxygen has long been known to affect the synthesis--the oxygen levels have been known to affect the synthesis of a variety of proteins in eukaryotic cells as well. We heard a little bit about what happens in erythropoiesis where certain cells in the kidney can increase their rate of transcription and synthesis, erythropoietin to work on the bone marrow to give you more red blood cells. Well, the same thing can happen for glycolytic enzymes, for example, perhaps for ion channels, and many observations have been made where cells will upregulate certain proteins in response to a drop in the oxygen tension.

What the overall benefit of that is may not be clear in all cases and what the exact sensor is is not clear

in all cases. We are very interested in the sensor and have been making some efforts to go after it. But as far as how the rest of this scheme might happen, once the oxygen system is sensed, how do you actually use levels of oxygen to regulate ion channel or other protein synthesis?

Well, transcriptional regulation in general is a huge topic, but one of the big advances in the last decade or so has been the appreciation of transcription factors which can bind to enhance sequences and turn on mRNA synthesis. And they can be activated in a variety of ways, either by some ligand like a steroid binding to them, by dissociating from a complex in the case of NFB, for example, by being phosphorylated, or by being newly synthesized, for example, cfos and cjun as we heard about earlier, which can go back into the nucleus and trigger another round of transcriptional activity.

The case that you heard about earlier from Dr. Hochachka about the hypoxia-inducible factors is a very illustrative case. In this example, somehow hypoxia is sensed by the cell. It triggers the cell to make a new protein, or actually it's a dimer of two proteins. The purification and sequencing of one of these proteins was just published about a month ago. This hypoxia transcription factor, you might call this the hypoxic immediate early gene, is a dimer of two proteins. One is about 120 kilodaltons, one is between 90 to 94 kilodaltons. One of them gets phosphorylated. One of them, this 94-kildalton protein, is actually identical to another protein that is a partner of the antigen hormone receptor that accompanies the antigen hormone receptor in and out of the nucleus, whereas the HIF-1 alpha, they call it, is a unique protein that has not been found anywhere else.

It has been shown that upon stimulation of cells with hypoxia there is de novo transcription of this protein, de novo translation, and then finally some regulation at the level of the final complex which requires phosphorylation that, again, regulates its binding to a particular enhancer that might trigger, for example, erythropoietin or some other protein.

Now, there are many steps along here that actually may be regulated by hypoxia as well in addition to the initial signal that kicks this whole thing off in the first place.

DR. SIESJO: Excuse me, just a question. When you talk about hypoxia here, is this an open system with a reduced oxygen tension, or is it a closed system like in ischemia?

DR. VERMA: Most of the studies have been done using an open system where all they do is they change the oxygen tension from about 20 percent down to anywhere from 2 to 1 percent to 3 percent.

DR. HOCHACHKA: Depending whether it's in vivo or in vitro.

DR. SIESJO: Yes. The reason why I ask is that many of these things can be induced by things which would accumulate during ischemia just because it's a closed system. You would accumulate the nitric oxide, hydrogen peroxide, a lot of things would diffuse out of the system which is open, and, therefore, they're working with only the oxygen tension.

DR. VERMA: Well, there's good evidence to suggest that it's particularly oxygen that regulates this activity, and there's good evidence along the lines of what Dr. Hochachka presented, that there's probably a heme protein involved in the sensing of oxygen at some point along this. But one point that I wanted to carry a little bit further, which Dr. Hochachka ended up on, was the fact that we should not think only in terms of this dogma of DNA, RNA, protein going in a linear fashion. Because concomitant with all of these unidirectional synthetic mechanisms, we also have mechanisms that are degrading all this phenomena. Once the proteins are made, they have a certain half-life and are destroyed in a certain time period, which includes transcription factors, which also is important for mRNA.

In fact, this is most important for the most rapidly inducible mRNAs. If you look at the literature, the most rapidly turned over mRNAs are those for cfos, cjun, TNF, IO1, GMCSF, all these immediately early acute responses. What has been discovered is that there is actually particular elements within the mRNA sequence that give that particular mRNA or make that mRNA have a very short half-life.

Now, if we look at mRNA's structure in general, typically in mRNA that comes out of the nucleus into the cytoplasm has various portions on it. It has a 5 prime untranslated region; it has a coding sequence. Then there's a 3 prime untranslated region and a poly A tail. It has been found that when mRNAs have a long poly A tail, that gives them a longer half-life, and the basis for that is actually the existence of a protein that binds to long stretches to poly A tails. So if you have a poly A tail that is about 30 or more, this protein will bind and will sort of fend off nucleases for a while to protect that RNA. If you have shorter than that, that protein can't bind. In fact, this protein has been identified and purified.

In addition, there have been several proteins that have been found that bind to sequences in the 3 prime untranslated regions. There are particular sequences here that can actually form stem loop structures to give you a double strand and nucleic acid structure, and these structures are very important for proteins to dock on.

When these proteins bind to these structures, again, they appear to keep these mRNAs from being degraded very rapidly.

The activation of those proteins, how those proteins are told to bind to the mRNA or not, is, I feel, a very important topic, and the regulation of that protein binding can happen very quickly. In fact, there have been several examples where mRNA levels are changed by a

particular phenomenon, particularly hypoxia. But transcriptional rates are not elevated that much. In other words, the steady state of the mRNA is increased without increasing transcription. That's implying that it's the degradation rate that has been affected.

Specific examples that involve hypoxia include: the EPO message itself, erythropoietin's increased the transcriptional level as well as at the mRNA stability level; tyrosine hydrolase in the carotid body, again, its message is stabilized by a protein that binds in response to hypoxia; the vascular endothelial growth factors, tropomyocine --there are about five or six--not five or six, but maybe about three or four others that I'm familiar with where there are certain proteins that get activated by hypoxia to bind mRNA.

This can happen really quickly and can change the levels of mRNA without you having to go through the nucleus, without having to have any transcription and so on. This can happen very acutely, and I'm talking like in one or two minutes at the most. In fact, only one of these proteins so far has been purified and identified.

So I think that this whole area is actually a broad are whereby changes in oxygen levels can actually regulate a cell's activity very rapidly and in a profound and long-lasting way. The same phenomenon or the same idea may also apply at the protease level. We know nothing about protein degradation rates and how they are regulated, whether there is also post-translation modifications of proteases. In fact, many of the nucleases and proteases that carry these out we have no knowledge about. So there is actually, I think, a big world besides the main dogma that we have been talking about that needs to be looked at with respect to rapid regulation by metabolism.

At the end stage of that, obviously, whatever the protein is determines the function of the cell, and in many cases these may be ion channel sub-units.

I think I will stop there.

COLONEL BELLAMY: Very good. Any questions?

DR. SIESJO: I think that was a very, very useful review of an extremely important area. I was thinking about what you mentioned, the ADP-dependent potassium channel. Actually, the remarkable behavior of the seals here and the turtles could, in fact, partly be due to, say, upregulation of ADP-dependent potassium channels. Then you can ask the question, Why should an ADP-dependent potassium channel change when you have no change in the ADP concentration? But I think that for some tissues it has been shown that these channels are actually regulated by the ATP/ADP ratio, and I'm talking about the free ADP now, and that there may be a much higher affinity for ATP than for ADP. So just by having a slight imbalance between ATP and ADP and a rising ADP, you could activate such channels. Then the membranes would hyperpolarize. That would make them less excitable.

If at the same time you get acidosis, you would reduce cation flux through the membranes. So that could really work as one really crucial piece of information, and I wonder if someone has it. What about the membrane potential?

DR. VERMA: I think these are all testable, and people have looked at membrane potentials in isolated cells. But as far as which cells have these channels and where they are found, I think that is--in other words, that directs what system you study. The thing is they are not ubiquitous. They are actually parceled out in particular areas.

A great example of that is that in baby rats there are not as many as these ATP-dependent potassium channels, but there's a lot more in the adult. In fact, there was a group that did a radiographic study with a ligand that binds to these channels, and they found they were predominantly in forebrain structures rather than brain stem structures. They were more in the rat than in the diving turtles. And they were more in the baby rats than the adult rats. They tried to relate that in some way to the hypoxic susceptibility of these animals.

But I think that the tools are there and the systems are there, and it is just a matter of asking the questions like you are posing.

DR. HOCHACHKA: Asking the functional question, yes.

COLONEL BELLAMY: Well, thank you very much.

Dr. David Lewis is going to talk next. Dr. Lewis, as I understand, to put it in perspective, the Advanced Research Project Agency has decided to fund an organization in Sweden to study the same question. So we have asked Dr. Lewis to report to give us some idea of what the European perspective is at present regarding suspended animation.

DR. LEWIS: Thank you very much for inviting me to participate in this very exciting symposium. I should mention that my brother is a lawyer, and he pointed out that bringing people to Washington in August is in the category of cruel and unusual punishment.

COLONEL BELLAMY: We all agree.

DR. LEWIS: I think you are in for a big legal problem here.

Anyway, as Colonel Bellamy pointed out, we have been looking into this problem. I will not be talking about research, but I will be talking about travels and about people and about questions that we have been asking.

My field has been in microcirculation, looking at physiological techniques in microcirculation that can be used to study shock and trauma. Before I start, let me just say a couple of words about microcirculation.

The things that people have been talking about today about microcirculation in anoxia and hypoxia have to do with adhesion, attraction of leukocytes to post-capillary

endothelium, but there are other important aspects of what can happen to the microcirculation.

The endothelial cell is a very peculiar cell in that it has a very large membrane, very large surface area for its volume. This means that it expends a great deal of energy in these things that we have been talking about, ion pumps and that sort of thing. So that when the endothelial cell becomes hypoxic, it has a very great problem in maintaining the integrity of its membrane, and you find very shortly after you induce hypoxia of endothelial cells, either in vitro or in vivo, that these cells start to swell. They have a problem in maintaining their volume.

The other thing is that when they change their shape, you can get gaps between endothelial cells. When the endothelial cell swells, because of the fact that it is attached to a basement membrane, the swelling means that it goes into the lumen, so you decrease the size of the lumen when you swell an endothelial cell. And when you produce gaps between the endothelial cells, then you also get an increase is permeability.

There is the question as to what part of the central nervous system is most sensitive. In skeletal muscle, we findthat the endothelial cell is much more sensitive than the myocyte. In the myocardium, I think maybe it is about 50/50; in the brain, the brain cells are more sensitive than the endothelial cells. But certainly the question about the microcirculation is very important.

Now, the motor behind all of this is somebody that many of you know, General Bo Rybeck. In this country he called him "Rye-back," but in Sweden he is called "Ree-beck." I got to know him because I was one of his faculty supervisors when he did his doctoral thesis, and he went from there to become surgeon general of the Swedish Armed Forces. In Sweden, there's only one surgeon general for all of the Armed Forces. And from there, he went to be director of the Swedish Medical Defense Research Institute.

When he retired a year or so ago, he was appointed by the Swedish Government to start a strategic research board, and this was patterned very much after ARPA here in the United States. The idea of this was to look at the whole spectrum of research activities, and one part of the spectrum was medical research. And because we had known each other for a very long time and I had been working in the area of shock and trauma, he asked me if I was interested. This started about a year-and-a-half ago when Don Jenkins came to Sweden and we began talking about this sort of thing.

that this is not an easy job and it is not something that one can solve in a year or so. What we see is a long-term project, looking at a whole spectrum of research from the cell, from the pieces of the cell up to the whole organism.

Our laboratory in Linkoping has a number of techniques using microelectrodes, also using metabolic techniques, looking at what goes on in the microcirculation. Two of my former graduate students have started a laboratory in Stockholm, and the most recent student will also be going to Stockholm. So I think we'll be moving most of the work to Stockholm.

There are a number of laboratories in Stockholm. The Defense Medical Research Institute has a laboratory. There is also a trauma laboratory being established at the South Hospital. This is a hospital built during World War II with an underground laboratory, built into the mountain on which this hospital sits. This is being used for courses in trauma surgery.

Lundis of interest to us because I have a very good friend there who is a documentalist. She is an expert in medical literature, has access to data bases all over the world. When we started this project, we gave her key words to bring out information. So in the process now we are establishing a medical library of papers published in this field.

One of the things that is in my favor is the fact that I am known to very many of the people working in this area. This came about because of the fact that during a 10-year period of time I was General Secretary of the European Society for Microcirculation, and got to know virtually all the people in Europe and many of the people in other parts of the world working in this area. I was one of the founders of the European Shock Society, so I know people working in shock, and Dr. Bazan and I were founders of the International Society of Pathophysiologists, so I know people working in pathophysiology.

I had the opportunity to be opponent on a doctoral thesis in Tromso, in Norway. This was very interesting because a group of people there were working in problems of hypothermia, Arctic research. What was of interest to me was that there were aquatic physiologists working on problems of animals on land and in the water in the cold environment; there were medical physiologists working on problems of mammals, including humans, in land and in the water; and then anesthesiologists and surgeons in the hospital working on problems of hypothermia, including accidental hypothermia.

I'm sorry that Professor Sorlie, who is chief of surgery there and the motor behind all this, could not be here today. I'm sorry he wasn't--

COLONEL BELLAMY: As you know, we attempted to invite him, but we were unable to contact him, at least with any ease.

We have talked about the effect of temperature, and we think here this would be very interesting from the point of view of the whole spectrum from the single cell to animals. We are also interested in the way that various

animals have "solved," adapted themselves to, various problems.

I'm sure many of you know more about this than I do, but the goldfish can survive frozen in ice. And he has a very interesting way of solving the problem. You remember the slide that Bo Siesjo showed, going from glucose down to pyruvate and then from pyruvate to carbon dioxide in water, and from pyruvate down, you need oxygen. If you don't have oxygen, you make lactate.

Now, the way the goldfish solves this, when he goes to pyruvate, if he doesn't have oxygen, instead of making lactate he makes alcohol. It diffuses very well in and out of the cell and also is a good antifreeze.

I have a very good friend who used to work in microcirculation in Moscow, and he now has a company that is looking into medical research and giving courses in medical research. We have asked him to look at Russian literature not published in English that has to do with trauma, hypoxia, hypothermia. The reason for this is when Russians publish in Russian, they get money for it; when they publish in English, they don't get any money. So they don't publish in English; they publish just in Russian. Much of this then doesn't get into the English literature. We don't know about it. So this is one of the things he's helping us locate.

The other is finding laboratories and research groups in the former Soviet Union that are interested in working with us on the problem of surviving severe trauma.

As you all know, there's a very serious problem in Russia. Many of the research scientists are leaving research because there is no money for salaries and there is no money for equipment or supplies. So if we can find people who are interested in this, we think we can employ them fairly cheaply to do research for us in this particular area.

In the Department of Physiology in the Semmelweis University in Budapest, there are four or five groups working on microcirculation in the brain and the effect of changes in blood flow in large vessels and what happens in small vessels. We have been talking to them about helping us in this particular thing.

Also in Russia, there is a group in St. Petersburg, one particular fellow who has studied in very great detail the changes in conjunctival blood vessels in trauma, and this he did during the war in Afghanistan. If you can't get into the brain in the intact individual, you might be able to get some information from the conjunctiva.

Colonel Bellamy talked about the European perspective. I have talked to the people that I know in Munich, in Cologne and in Belgrade, and hopefully when we get some money, we will start research. But it is not just the European perspective because I also have connections in other places in the world. For example, we have borrowed a machine from a professor in Tokyo who works at the Medical and Dental Research Institute, which non-invasively measures the

temperature of the brain and compare this with temperatures in other areas. This is why I was interested in the relationship between brain and core temperature. We have started doing this on children with malaria in Africa and hope that with more experience to be able to apply it to this particular research project.

We have talked to a number of groups in Singapore about working with us. There is a group in the Institute of Cell Biology that is interested in how trauma to a cell will affect signal transduction. We think this may be a very important component of things that are going on. There is a fellow there working on robots, and this is another interesting aspect. If you don't want to send a corpsman onto the battlefield, maybe you can send a robot out to do the job. This is also something you have talked about that ARPA has been doing.

Well, there are many other aspects. I think the point of this is that we are very much interested in this particular area. We are trying to explore over a broad spectrum of the literature to find people who, with us, will be interested in doing research in this area. It will take a long time to do it, but we think we should start with the cell. We should study organs, individuals, and also apply this to man.

Thank you.

COLONEL BELLAMY: I would be curious to know what key words you pick to do the literature search.

Secondly, in our experience, we have--in my group is a former Russian diplomat who is also a medical doctor and is very interested in research. We have made some effort to try to ascertain what is in the Russian literature over the last 20 or 30 years that would be applicable to suspended animation. The material usually is presented in a way that I cannot judge whatsoever. I can only say that I wish you well if you are trying to ascertain what--

DR. BAZAN: The controls in their experiments, which type of controls they have run in their experiments and experimental design, we have run into some problems recently with a paper, then after looking at it carefully, the way that the controls were made and the experimental design was set up, it was--

COLONEL BELLAMY: Didn't have any controls. That's one of the problems.

DR. BAZAN: Well, I--well, yes.

COLONEL BELLAMY: You didn't want to say that.

DR. BAZAN: Yes.

COLONEL BELLAMY: There is an awful lot of material there. The question is--it is very difficult to determine what it means?

DR. LEWIS: Yes, the point is--I don't know what we'll get out of this, but hopefully something. We think it

is at least worth the effort since we have somebody that can help us do this.

As far as key words are concerned we have used oxygen, anoxia, hypoxia, hypothermia, hemodilution, hibernation. I don't have the whole list here.

COLONEL BELLAMY: Yes. I was just curious. Clearly it would be very important to try to identify--it would be useful for everybody to know what at least one group thinks are key factors in this whole picture.

DR. LEWIS: Yes. The problem we have experienced so far is that you get out 100 papers, and there may be one or two that are worth following up.

COLONEL BELLAMY: Yes, that's right.

COLONEL BELLAMY: Well, let's stop at this point and have a break. Then we're going to come back, and Commander Yaffe is going to tell us what the Navy plans for this area, and then we will try to sum the meeting up.

COLONEL BELLAMY: The work being done by this group and the work having been done by the previous group in February will not be wasted. It would appear the Navy has interest in making this whole group more formal, and also carrying out a research program which in fact might bring this project to fruition. Although as I pointed out to Commander Yaffe, they should insist that his group not be required to make a report as to their progress for at least 10 years.

Commander Yaffe's going to tell us what Navy plans are.

COMMANDER YAFFE: I'm sure the investigator would like that, not having to make annual reports. I think, in general, there's a growing interest in this, not only on the part of the Navy, but also on the part of the Army, not that there hasn't been a continuing interest on the part of the Army. But I think together we're going to solidify more of an interest, which I guess in real terms translates into dollars put against some of these ideas.

I'm here, as I said earlier, from the Naval Medical Research & Development Command, which is in Bethesda, on the same base as the Uniformed Services University and the National Naval Medical Center.

And we're a command with about seven laboratories, though, unless I call in to the office and doublecheck, it can change on a daily basis.

Just as an aside, it's like this week, our laboratory in Cairo, because the officers and the U.S. citizens who work at that laboratory don't have diplomatic immunity, there are several lawsuits going on where the Egyptian courts feel they can make the Navy pay, whatever the particular case is.

But the ambassador, our ambassador to Egypt, has decreed, that unless we're given diplomatic immunity, the U.S. Government is going to close that laboratory.

So that's being decided, you know, probably by mid next week, and we might have more investigators working with NMRI a couple of weeks from now than we do right now.

But we do have a series of laboratories around the world, and our largest is in Bethesda, the Naval Medical Research Institute. And under the Base Realignment And Closure Commission decisions, or the BRAC, that laboratory is going to be closed and consolidated at Forest Glen, where the new Walter Reed/Inouye Building will be constructed as a combined Walter Reed Armed Forces Laboratory, at least combined Armed Forces Laboratory from the Navy's perspective.

I'm not sure, exactly, what the Army thinks. I guess it depends on who you ask. But there will be--right now, the Army, Navy, as well as the Air Force, are working together on implementing a plan for the Armed Forces Medical Research and Development Agency, which will be, in a sense, sort of a tri-service activity with as much co-location, including headquarters, working together on military medical needs. And in one sense, at Walter Reed, we'll all have a brand-new facility in that.

That consolidation concept, together with the fact that there's general downsizing in the Defense Department and tightening of the budget, it's sort of a time when there's a fair amount of chaos, which makes it a time when one can, if you're aggressive enough, can implement changes more rapidly than you can during the normal course of events.

So now is a very good time, I think, in the military to come forward with interests in, which we can call life sustainments, suspended animation, or pickling, as Dr. Safar says.

Because those concepts, even though the military hasn't pursued them aggressively, are really fundamental, as Colonel Bellamy has said, to what we're supposed to do in casualty care, primarily, and that is save the lives of those individuals who are wounded.

And while we say it may be difficult to approach that logistically in the first five minutes, if we don't begin with the science, of course, we'll never have the opportunity to figure out, you know, how to implement it on the battlefield.

I guess it's about two and a half years ago, I was down at the Pentagon, and I tried to get some interest going in this, along with some interest in producing a more focused program at the Naval Medical Research Institute in blood products. And as things would have it, also for fortuitous reasons which I won't go into, at the Naval

Medical Research Institute we have brought in a large group, now to work in blood products, which is being headed up by Dr. Harold Merriman who just recently retired from the Red Cross after 25 years there. Prior to that--it's funny--he was at the Naval Medical Research Institute and left to go to the Red Cross. So he's come full circle now, and we're working a little bit with the Army group that's co-located with us.

And that's become a fairly large group, which interestingly enough is not only interested in what some people feel are mundane issues like extending the refrigerated shelf life of liquid red cells and, perhaps less mundane, dealing with frozen red cell problems or how to get platelets out in the battlefield; but that group also has a very strong interest in cryobiology, because cryobiology is fundamental to the preservation of blood products, opportunities for lyophilization of blood products.

So some people in that group also have an interest in its applications in life sustainment, and they do work with some companies on some proprietary approaches to actually already, at these companies that they work with, do some of these dog experiments, where they keep dogs in a suspended state for a number of hours, as well as they look at the effects of cold temperature on protein stability, and conformational changes that are irreversible at low temperatures.

So that's all in the blood transfusion medicine group, but I think there's going to be a strong overlap between that group and, let's say, the new initiatives which we hope to press on with beginning this October, which is the start of the new fiscal year.

And several of the investigators from NMRI are here to look at what the potential aspects, and see where their current expertise meshes with this, and how they can, from the in-house perspective, augment the activities.

And ultimately, what we would like to do, as quickly as possible, though obviously it will be some time in the next fiscal year, is to actually bring in a significant number of people, which we do by a contract mechanism, as well as perhaps some added leadership experience in these particular areas, to have a strong in-house capability. It's very important, particularly in the climate of downsizing now, and a lot of factors as far as in-house work versus extramural work, to have a strong in-house capability, and I think together with the Army and what will happen in the new Walter Reed, it becomes particularly strong for us, Army, Navy, to stand together on some of these major issues.

But to also have that in-house program tightly coupled in a collaborative way to various biotech firms and university efforts in this area, so that we can have more

visibility, more credibility, more support for the general concepts here.

And I think DoD, or our in-house laboratories, together with Uniformed Services University and other DoD performers, like the AFIP, or whatever, with civilian universities and biotech firms, we can really sort of create, I think, a national interest in this, and focus as a basis for building a stronger input for funds. That's the hope I have, you know, for the next couple of years, anyway, to really significantly get this off the ground.

I mean, the Navy is committed to putting in a significant amount of money in these areas, but obviously, it will take much more than we can do on our own.

Currently, with Steve Bruttig and Bill Wiesmand, who both are the casualty care counterpart in the Army, we've been meeting weekly, in order to establish an approach, an initial approach to what we feel are some of the priorities, and very shortly are going to form five working groups that will be configured primarily of key investigators, in-house and extramural, to sort of help us put together some documentation and strategies as we move forward to sort of more effectively sell these programs at a DoD level.

With the tightening budget, the military is emphasizing much more that we work on defined needs and requirements, and almost this year, for the first time in my recollection, the director of Defense Research & Engineering, that office has come forward with really--I mean, some people don't agree with it--but at least clear guidelines about programs that they think should be absolutely stopped, regardless of the quality of those programs.

In fact they've recommended stopping some programs that the labs feel are their highest quality programs.

COLONEL BELLAMY: Could you elucidate? Can you tell us what the programs are? That's very interesting to know.

COMMANDER YAFFE: Well, this hasn't been officially released, but they have recommended that we not continue our efforts in transplantation. They recommend that we discontinue the work in military dentistry, work in bone marrow stem cells.

The reason they're doing that, you know, not that it's, let's say, a final--it may be a final decision on their part, but it doesn't mean that there's not some maneuvering room. I think the reasons are because with tightening budgets, they say, number one, it must be a defined, clear military requirement, and you know, people always say, Well, that's a changing point.

And also, besides that, they want the efforts not to be substantially done by the civilian community. In

other words, if we're just a drop in the bucket compared to what's done in the civilian community, they wonder why we focus on that.

In an area like transplantation and immunosuppression, they say, Well, there are massive efforts in that, and so we have to strategize about those programs to see what we're going to do, because there's a group of investigators involved.

At the same time, we press forward with things that have a clear long-term requirement, and I think this clearly is one of those areas.

It's also interesting to me, because Dr. Hallenbeck is here, who used to be part of NMRI, as everyone particularly knows, particularly the diving community, which is undergoing substantial change now, because the Navy is moving the main diving activities down to Panama City, leaving the basic research, or what's called the science and technology portion at NMRI. And that cuts their budget in half.

And some of the things that were mentioned here today, some of the models that are used, you know, really relate, in a sense, to diving, and understanding some of the needs of diving. So I think this work may also have a spinoff to assist the investigators in diving, that would be left behind to perhaps find additional funds and add their expertise to this.

COLONEL BELLAMY: You realize that this meeting came about primarily because of the diving program. Dr. Hochachka spoke to one of the scientists there; I was interested in the effects of inhalational gases on membrane conductivity and just happened to talk to her about it. That's how we got involved in this.

COMMANDER YAFFE: Right. There is a strong relationship there, that could be developed, and of course my being invited to this, and NMRI investigators coming, is also, in a sense--I was sort of beginning to work more on this track as I came to the R&D Command, and Captain Walter, who a few months ago was the CO at the Naval Medical Research Institute, when Colonel Bellamy was there to give a talk, and Captain Walter recommended he come and talk to me, because he knew that I had an interest in funding these things.

And then we sort of got involved, and I found out about this meeting, which was, in a sense, fortuitous, and fortunate, before I sort of advanced on these fronts and repeat what had already been done from sort of an organizational standpoint.

So that's really the perspective for the Navy. We're strategizing about how to begin this as an in-house program, you know, selecting those areas that we feel are most critical, and I think we're all benefiting from the discussion here, and we want to initiate those programs with

some of our in-house staff, and also bring in additional investigators. And then also see where we can collaborate with some major centers that have a similar interest in this and are able to help us in both the short and long runs with this.

COLONEL BELLAMY: I have a worry about the question of how you describe this program to the funding agencies downtown. I'm concerned that if you go to them and say, We're interested in carrying out research in suspended animation, they may well laugh at you, and just say, Science fiction, you're wasting the taxpayer's money.

Does anybody have any idea of whether this a problem?

MAJOR BRUTTIG: Yes.

COLONEL BELLAMY: Yes; go ahead.

MAJOR BRUTTIG: Yes. I can tell you that from the perspective of the people downtown, they pay very close attention to what the Army science and technology--in the case of the Army, Army science and technology master plan says, because that--

COLONEL BELLAMY: Which of course is based upon, by and large, directions produced by amateurs.

MAJOR BRUTTIG: That could be.

What I'm getting at is that it's a planning document, and it becomes incorporated at a lot of funding levels by people that are not close to the science--

COLONEL BELLAMY: And blind lead the blind.

MAJOR BRUTTIG: In this case, the Army and the Navy are going to consolidate in many of their research efforts, and the Army specifically has, for several years, a documented listing for both hibernation and suspended animation.

COLONEL BELLAMY: Oh, they do?

MAJOR BRUTTIG: Yes.

COLONEL BELLAMY: They actually refer to it as "suspended animation"?

MAJOR BRUTTIG: Yes, because I'm the one that put it in there, and--

COMMANDER YAFFE: And coming out of DDR&E's office, these recent directives include aspects of dealing with exsanguination, ultra-profound hypothermia, and so there have been a lot of, let's say, buzz words that have been put in.

What you say is true. You can't just go down there and say, you know, We want $5 million to do suspended animation work. It's a weekly process, and in some sense, talking to each person that you have to talk to, slightly

differently, and building a consensus for what you ultimately want to do.

But I think it's certainly a doable task.

COLONEL BELLAMY: And do they realize that this is a problem primarily in basic science, at least initially, at least for 5, 10 years? Do they realize that?

COMMANDER YAFFE: Well, again, it depends who you talk to. If you talk to people at the Office of Naval Research about it, they're most interested in the basic science aspects.

If you go to speak to one of our admirals about it who's in charge of our more advanced pots of money, you know, they're more excited about, you know, showing how there's some animal that can survive without oxygen for six months, and here's a possible way we can make that happen in humans.

COLONEL BELLAMY: "We feel that in 15 days", or something like that.

COMMANDER YAFFE: But you do have to take a different approach with the numerous people that you have to sort of lobby with at the Pentagon.

You know, it's gotten to the situation for medical R&D, in the military, that the congressional earmarks actually almost--correct me if I'm wrong--let's say are equal to what we should call our "core budget," meaning our core budget that's given to us to execute on what are the defined combat, military-relevant operational problems, from infectious disease, nuclear, chemical, biological, medical defense, to combat casualty care.

That's, in a sense, dwarfed by money that's given for breast cancer research, prostate cancer--a long list of things. Not that those issues aren't important, but Congress, for whatever reasons, decides they can plus-up the DoD budget. But I mean, ultimately, these issues become just as important as those issues to the general community and also, very clearly, to the military. So I think if the game is played correctly, this program can have a very long lifetime.

In the Navy, I was at a meeting last week with the people who are involved in what's really a 35-year program for building the next generation--they call it the 21st Surface Combatants, and it has to do with our warships. And they're going to be smaller, maybe 30 to 40 percent smaller in size and, not that I understand the details, faster and much more lethal in their capabilities. And the manning for these ships will also be down by about 50 percent. They'll be much more heavily automated.

But as an aside, for combat casualty care, the ability to care for casualties on those ships will be much more limited because of the manpower. And so being able to deal with a casualty in a suspended animation way for those

ships makes much more sense than having the facilities to fully resuscitate them, because there's not going to be the complement of crew to do that, and there's not going to be the necessary facilities on board.

That's sort of a sellable approach from the Navy's perspective. And we are also responsible with the Marine Corps, and it's very similar to what the Army's requirements are. But in a sense, uniquely for the Navy, you're not even going to have the luxury of saying, Well, we can medivac them out; maybe, you know, we can do that in the first 15 minutes. For Navy surface ships, there's no place to go, and if you have casualties on a ship where you don't have the ability to resuscitate them, maybe there will be one day some simple pharmacologic approaches where you can, you know, suspend their life functions for half a day until you can evacuate them.

COLONEL BELLAMY: So they have scientific credibility; otherwise, it's--

COMMANDER YAFFE: Well, that's true; in the long run.

COLONEL BELLAMY: We can't give the line the idea that we can do some remarkable thing like keep somebody in a suspended animation status for days

COMMANDER YAFFE: No, no. That's not my intention.

COLONEL BELLAMY: You're talking about an hour, or a half an hour, or something like that. But again, it's got to have scientific credibility; otherwise, ultimately, everybody will look upon this as being another governmental boondoggle.

COMMANDER YAFFE: I think there's clearly data that demonstrates there's a potential in all of this. Whether it's initially extending an hour, or six hours, you know, who knows yet? But this is not--at least I don't feel it's total science fiction anymore. There's clearly things that are done clinically now in hospital settings that are characterized as life suspension for an hour or so. I mean, you all know that.

So it's not a matter of trying to sell science fiction to the military, to the admirals and the people that control the money.

I mean, usually, at least the line admirals are usually very bright individuals. But it's a matter of laying the story out in a scenario that makes long-term sense to them, with some credibility, as you say, and then usually you can be successful.

COLONEL BELLAMY: Well, let's complete our session. There are two basic questions that I think need to be addressed. One is, as Commander Yaffe just said, the scenario. I think it's absolutely essential that a clear-

cut, common, realistic, combat casualty care scenario be framed.

DR. LEWIS: Can you go stand there now?

COLONEL BELLAMY: Sure.

The basic scientists have to realize this is the ultimate goal, clear-cut, well-defined clinical scenario, which I think we want to discuss. Dr. Safar and Dr. Bruttig and I want to discuss this so we have some unanimity of opinion.

The easy part of the program, of course, is to tell the Navy what they should include in their research program, what they should do in the way of protection, preservation, and resuscitation research. I'm sure we all have ideas.

But before we start that, we want to address several questions to Dr. Hallenbeck regarding the response of his animals during hibernation.

I think it would be fundamentally very important to know whether or not there exists some sort of molecule or molecules that cause or induce the hibernating state. Is there a hibernation trigger?" What if one had a hypothetical hibernating tissue and measured its metabolic rate at the normal temperature of that particular organ, and then infused or administered the hibernating trigger? What happens to the metabolic rate with the temperature constant?

Would you please talk about that? Dr. Bruttig I think also has some ideas regarding this question as well.

DR. HALLENBECK: There have been a number of efforts to isolate a hibernation trigger of some sort, and I think in your last meeting you discussed--one of the people who--

COLONEL BELLAMY: Dr. Oeltgen was here and spoke about that, yes.

DR. HALLENBECK: He's somebody who's written a great deal about that, and there are others who have also tried to isolate this sort of a trigger. In these sorts of efforts where you use plasma and you try to fractionate it, or you use an extract of brain or something of that sort, it has been pretty elusive to come up with something that was really convincing, you know, that it was truly the trigger.

And I think that it's probably going to take a very systematic study to come up with the regulating mechanisms, along the lines of the things that have been done in the turtle.

But as to whether there is something which persists, if you take, for example, a preparation up to 37 degrees, and the effect of hibernation persists when the dimension of cold is removed, that has been seen in a number

of instances. The succinate-supported respiration in mitochondria is one example I can remember.

Mitochondria from hibernating animals will, even if it's warmed up to 37 degrees, still show a considerable block in succinate-supported respiration in mitochondria.

So there's something that does persist, that is in the--

COLONEL BELLAMY: Because my assumption was that in the hibernating animal, the substance dissociated the normal body temperature control from the ambient temperature; so if the ambient temperature is lower, the metabolic rate would fall, because it's no longer coupled with the temperature. But that's not the case. There is actually downregulating of metabolism.

DR. HALLENBECK: That's the way it seems to be, and in terms of the rates of things, as the animals cool down, the decrease in oxygen consumption seems to outstrip the fall in temperature.

So although it isn't--how it's done is not known, the general feeling I think is that this is not a kind of a retreat, I guess, to primitive poikilothermy, is the way I've heard it put. In other words, just giving up and saying I'm no longer going to resist this cold temperature, I'm just going to let myself get cold.

It's not that at all. There is some sort of systematic way in which everything gets brought down in a balanced sort of way, so that there's a new set point.

COLONEL BELLAMY: Good. Excellent.

DR. SIESJO: The question must be what is really causing the protection, and one wonders if that isn't the reduction in temperature. So you need your signal not to change your membranes, but to downregulate temperature without getting into a stress response or something.

It's very well known for brain ischemia that if you reduce metabolic rate by lowering body temperature by 10 degrees, 37 to 27, you have a tremendous protection against ischemia.

If you take barbiturate anesthesia, reduce metabolic rate in the brain by exactly the same amount, you have no protection. So you reduce metabolic rate without reducing temperature; then you see no protection. But if you reduce both, then you see protection.

COLONEL BELLAMY: Dr. Safar wants to talk about this, I think.

DR. SAFAR: This raises a topic which we haven't heard much about, and this is the difference between active and basal metabolism primarily in the brain. An anesthetic can push the brain metabolism down to half normal, with EEG activity zero, but not below that. The remaining oxygen

uptake is required to keep viability of cells going. This is called basal metabolism.

We know we can get active plus basal metabolism down to near zero with profound hypothermia. My question is, can anybody think of a chemical way to do that? During cooling it can be counteracted if we don't produce poikilothermia. This was achieved clinically, in the 1950s, by Laborit of France, using a lytic cocktail. This is a form of pharmacologic hibernation.

Questions to you 1) In hibernating ground squirrels, why does the heart continue beating below 20 degrees when all non-hibernating animals' hearts just stop?

Many dog hearts stop not always in fibrillation, sometimes in EMD. They may fibrillate or not, as you get the temperature down it depends on the anesthetic. They finally stop. The EEG can keep going, very slowly, at a very low temperature. This is remarkable.

DR. HOCHACHKA: By the way, do they beat regularly, or--

DR. VIRMANI: Irregularly.

DR. HOCHACHKA: Irregularly, like, you know, three or four beats at a time, then a long bradycardia, then ding-ding, and then--

DR. SAFAR: Chain Stoke's beats.

DR. HOCHACHKA: Yes, like chain stokes breathing, is this chain stokes heart rate? Is that the way it is?

DR. SAFAR: It will still beat.

DR. HALLENBECK: It's not as regular as usual, but it's not--I mean, there's some irregularity.

DR. SAFAR: But they are not in cardiac arrest and they are not apneic. So they are not totally anoxic in their blood. There is a minimal blood flow in hibernation. This is from suspended animation.

Hibernation applies to the controlled or uncontrolled hemorrhagic shock state, when we want to keep the low flow going to prevent wrecking of organs. That is hibernation.

In suspended animation during total circulatory arrest time PO2 ends up zero. All stops.

DR. SIESJO: Why should you have any general effect of a 10 percent blood flow if your temperature is 7 degrees under, if you have no circulating PMNs which could block the--I mean, the tissue is not energy-deprived. You have 10 percent blood flow--

DR. SAFAR: That's what I mean.

DR. SIESJO: --and you have a temperature of 7--

DR. SAFAR: When the circulation stops completely, you are talking about anoxia, eventually.

DR. VIRMANI: But you don't have to stop and do that.

DR. SIESJO: No.

DR. VIRMANI: I mean, there's nothing that tells you--

DR. SAFAR: We are talking about suspended animation, the exsanguinated, pulseless organism. And trying to differentiate in our thinking and planning between when there is low flow or trickle flow in the shock state, on one hand for that hibernation is appropriate -- and cardiac arrest on the other hand. That is suspended animation. These are not the same thing.

DR. HOCHACHKA: Let me just say a couple of quick things about hibernators that I think are unclear. First of all, a hibernator is not a poikilotherm. He regulates his body temperature at a new set point very, very precisely. He's a homoiotherm. He's normoxic. There's no evidence of any, you know, oxygen limitation.

And the final point is that when metabolism is carbon fuel, plus oxygen to CO2, water plus heat, and it makes perfect biochemical and biological sense that metabolism be depressed, and as a result of that--that's the furnace, you've turned the furnace down. Then you measure the consequences, the body temperature, and it goes down.

You want to warm up; you turn the furnace on. Then the body temperature comes up. So it makes sense that body temperature track behind metabolism.

So it's metabolism that's being regulated. It's not temperature, some weird way, trying to regulate--I mean, temperature regulating the metabolism from the outside, as in ectotherms.

DR. SAFAR: What are tissue PO2s in the hibernators? And what's the pH in vital tissues?

DR. HALLENBECK: The pH can be measured at--we've measured it at various levels. We've gotten it as low as about 6.9, but in others--this is if you figure what it would be at 5 degrees.

But at other times, it's been normal, the blood pH. The most recent paper we did, it was normal.

DR. SAFAR: Yes, and the tissue PO2?

DR. HALLENBECK: But as far as PO2--

DR. SAFAR: Tissue PO2.

DR. HALLENBECK: Yes. I don't know about tissue PO2. The arterial PO2 is interesting in the animals,

particularly when they're awake. We had been accustomed to not wanting to use animals, you know, if the PO2 was much less than 90 in rats. You tend to feel something's wrong if it starts to get much below that.

We were seeing awake animals that had PO2s of 45, for example, and they looked fine. They were bright-eyed and so forth.

We started to talk to people, and the general feeling was that all mammals are the same; they need to control the PO2.

And then we found some statements--we had trouble tracking this down, but it turns out that so-called fossorial animals--this really doesn't have to do with the hibernating state--but animals that go into burrows and plug the burrow, and then they start breathing down the oxygen and breathing up the CO2, have got to be able to tolerate wide ranges of PO2.

And these animals are kind of indifferent about where their PO2 is. It's astonishing. You can imagine what you do if you get a PO2 of 45. You go through multiple machines, you know, and there are a lot of questions, but they all gave the same reading. So they're kind of casual about where their PO2 is.

DR. HOCHACHKA: Can I just sneak in one more quick question there? I mean a comment. Andre Milan recently, I heard him give a summary of the pH stuff, and what he finds is, some tissues are actually made acidotic by CO2 retention in the hibernating state, and he thinks it's sort of like what we were discussing before, the possibility of hydrogen ion being used as a negative modulator--

DR. SIESJO: Right.

DR. HOCHACHKA: --and you know, kind of forcing, depressing metabolism. And in the brain, especially, he made a big, big point of saying that pH was absolutely normal, you know, it's just where you'd expect it for a intracellular pH very close to--

DR. SIESJO: That's in support of alpha stat.

DR. HALLENBECK: The old alpha stat story, exactly. So some of them, you know, follow the alpha stat, and therefore maintain a normal pH, some of the tissues, but some, especially skeletal muscle, definitely, they retain CO2, go acidotic, and he thinks that's a key depressing--a metabolic depressant mechanism.

DR. SIESJO: It's a good thing.

DR. HALLENBECK: Yes, it's a good thing. They're doing that intentionally. So their RQ's go strange while--you know, for time periods, but when you get them to new steady state, their RQ is .7, which is what you get for fat oxidation.

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DR. SIESJO: We don't know how the hibernator's brain is to ischemia. Suppose that you take a hibernator at a temperature of 5 or 6 degrees. You induce ischemia of sufficient length to give you cell damage. And then you take a rat and bring him down to 5 to 6 degrees and see if the damage is worse or not.

So my question is, Is there anything in the hibernating sequence which will make membranes more protective, as you see it in seals instead of trauma, or is it just that the hibernator has a means of turning off heat production, and then temperature goes down, without any problems with cardiac irregularities, and then also with sequestration of the formed elements will not accept erythrocytes?

DR. HALLENBECK: It's very hard, in vivo, to do this, I mean, because if you take even a ground squirrel, which you would think would be the sort of animal that would be resistant to cold, and just artificially cool it when it's not hibernating, the prep--you know, if it's anesthetized, and you're going to do surgery on it, and so on, it just deteriorates very rapidly. So that there's this additional stress of cold, of that much cold on the animal, and so that's been hard to do.

We've thought of doing things like having total ischemia by decapitation, then warming one head up and looking at it. We've looked at MAP 2, for example, which is a microtubule-associated protein that you can stain, and you can look for cytoskeletal things disrupting in the cells, compare it with rats, and I mean, the hibernators go a very long time without any disruption.

The other thing that has been seen is that if you do tissue slices and things of that sort, hibernating brain will last much longer than non-hibernating.

COLONEL BELLAMY: At the same temperature?

DR. HALLENBECK: At the same temperature. So when you control--

COLONEL BELLAMY: So there is a protective effect on the--

DR. HALLENBECK: Well, I mean, the evidence is not as good as you'd like, but I mean, these are the things that bear on the question.

DR. SIESJO: I think this is very important, what you're saying. A German group worked many, many years ago on extreme, 100 degrees, hyperthermia. They found that when they came below 10 degrees Centigrade, they actually saw a loss of ion homeostasis.

So they concluded that the effect of the temperature lowering on ATPase's was larger than the effect on ion conductances. So it could be that the hibernator has a means of regulating membrane permeability, so that you

decrease conductance, now you are decreasing energy, so to speak, in sodium transport would be matched.

COLONEL BELLAMY: That would be a very important question to answer. Clearly, that would be a very important question.

DR. LEWIS: Can you get an isolated organ to hibernate?

DR. SIESJO: Good question.

COLONEL BELLAMY: You said yes.

MAJOR BRUTTIG: Yes; yes. That is an excellent question.

COLONEL BELLAMY: Who knows the answer?

MAJOR BRUTTIG: Well, no, I don't, but go ahead and answer it and I'll jump in when you--

DR. HALLENBECK: I mean, unless you--I don't know how Oeltgen presents his material, you know, on the isolated organ prep, and so forth, if he's thinking of that as hibernating. But he protects it, I guess, with--until you know what controls things, I think it would be hard to do. The best you could do is just take it out of a hibernator, and then study it at the same temperature, or something.

Did you have another comment?

MAJOR BRUTTIG: Well, I have several comments, but the first thing to acknowledge is the fact that we're putting Dr. Hallenbeck on the spot. We could spend two weeks trying to set the proper perspective for where hibernation fits in as a survival mechanism relative to things like estivation, which was also mentioned a little while ago, and we haven't talked about torpor, which is another mechanism sometimes employed by non-hibernators, sometimes employed by hibernators, et cetera. And then there are overlying biological phenomena--acclimation, acclimatization, and adaptation--and we haven't put those in perspective either. And so he's going to have a difficult time trying to answer those to a group that maybe isn't thinking in those terms.

But to get to what my answer would be specifically to the question that was posed to you is that Dr. Oeltgen's group has a subcontractor--my terms--a collaborator, who is, I think, at Mayo. His name is Steve Bowley, who has a standardized prep--it's a Langendorf heart prep, so he's studying cardiac mechanics, and most often he uses the rabbit.

So he was asked to study the use of the hibernation induction trigger, which is what Oeltgen calls this fraction that he isolates from plasma that's associated with albumin, on its effect on the isolated rabbit heart contracting in the Langendorf prep.

Now, what Oeltgen is interested in is ischemic hearts, arrested hearts, and then trying to salvage myocardial function with the resuscitation of the organ.

And so when he first did his studies using the hibernation induction trigger, there were results that were all around, up and down, but it looked like there was some promise for this hibernation induction trigger in, one, protecting the heart from ischemic arrest and returning myocardial function. But it turned out that the spurious nature of the results was probably due to a batch effect of the hibernation induction trigger.

They cleaned that up a little bit, and he seems to have much tighter results now. He can return the hearts to function after, I think it's an hour of ischemia. It's quite a long period of ischemia, and he doesn't get a 100 percent return to myocardial function, but he gets about a 70 percent return if he uses the hibernation induction trigger.

And without the hibernation induction trigger, you get about a 30 percent return in contractility, in myocardial function.

So there is some protectiveness, and we're talking about an animal that doesn't hibernate, but using a hibernation induction trigger derived from, in this case, groundhogs, woodchucks.

Not touched on here is also a species effect, and one of the things that I've mentioned to a couple of people, just in passing, is that we tried to get some people at Temple interested in this, because they're interested in both brain injury and spinal cord injury, and we were looking at a way of salvaging the tissue until you could do something else.

Well, they were really interested in checking out this hibernation induction trigger, which Oeltgen seems to attribute to a delta opiate agonist function, and the reason is because he can block it with Meloxone and Altrexone which are opiate blockers.

And so he made available the hibernation induction trigger, and one of the delta opiate agonists, and they tried using this in the rat, and there's absolutely no effect on the rat, conscious or unconscious, repeated doses, et cetera. So there are animals that, for one reason or another, are resistant to this.

Now, the one thing that worries me about experiments like that is that it might lead someone to the conclusion that if you're a hibernator, these triggers work in hibernators; if you're a non-hibernator, these triggers don't work in non-hibernators.

And there is evidence from other sources that when you give these hibernation induction triggers to primates,

you can cause not hibernation but a harmonious slowing of physiology, biochemistry, et cetera.

And there's the real trigger. When we talk about cooling an individual, we're talking about a physical effect on a biological system. We're talking about a Q10 effect. We're talking about maybe a membrane effect, as Dr. Hochachka and I were discussing, with respect to cold, et cetera, as opposed to harmonious control over physiology which has been the long-term evolutionary adaptation of this kind of animal. So that he's set to do a lot of things in response to the environmental cues that he gets, and then gives out this trigger.

So it should be no surprise at all that the tissues recover fully. It should be no surprise at all that he doesn't have ionic problems. It should be no surprise at all that the PMNs are parked, if that's what they're doing, et cetera, et cetera. In essence, they have worked out all the problems.

It's not just animals like ground squirrels that do that. Certain bats do that, and animals as large as bears do that, and they range in time for how long they can hibernate, from anywhere from a few days to a few weeks, to months at a time, without true arousal. So there's a very large span of response, and, quote, "promise of protection."

COLONEL BELLAMY: Dr. Bazan has a comment.

DR. BAZAN: Actually, I don't know where the story of the brown fat of hibernating animals is nowadays. I would say the project that I had as a medical student during two years in Rome many, years ago, at that time there were substances coming out of the brown fat which is highly developed in hibernating animals. Particularly one. There was an uncoupler, phosphorylation from the respiratory chain. And the hypothesis was fuller --I don't know where the story is now--that these substances could regulate temperature and metabolism.

COLONEL BELLAMY: Well, raise body temperature, of course.

DR. BAZAN: There were a couple of papers at that time, showing brown fat in the newborn, I mean, the human newborn, and I wonder if the search for a hibernator trigger, or triggers, have continued along those lines, because there might be a linkage between the brown fat of the newborn and the brown fat of the hibernating.

COLONEL BELLAMY: Well, it is my understanding of brown fat that its mitochondria are so set up that there is an uncoupling of oxidative phosphorylation, and production of ATP. The energy is lost as heat, and this is a way of maintaining body temperature rather than a hibernating effect.

DR. BAZAN: But how factors from the brown fat that will go into the bloodstream, like the--

COLONEL BELLAMY: I don't know if there are factors.

DR. BAZAN: Are there any--yes?

COLONEL BELLAMY: Well, Dr. Hochachka could tell us. Unfortunately, he has stepped out.

DR. BAZAN: Yes. I have not followed the literature, closely.

DR. VERMA: The uncoupling protein has been isolated. It's a 30 kilodalton protein.

DR. BAZAN: And it goes into the bloodstream?

DR. VERMA: No. It's actually an inner membrane protein, but it has marked sequence homologies to the ATP/ADP translocator, of which there's three isoforms. But it seems to form a channel that shortcircuits--and whether other cells can express this protein isn't clear. It hasn't been mapped by in situ hybridization.

DR. SIESJO: Do you know if this is similar to the pore described by Cronto's lab?

DR. VERMA: All those proteins are very similar. Well, that protein has been isolated, that pore. But there is another pore that I mentioned in my diagram, which is very similar in sequence to the ATP/ADP translocator, phosphate carrier, the citrate carrier--they all have a lot of biochemical properties and marked sequence homologies.

DR. SIESJO: Do you know if it's sensitive to cyclosporinate?

DR. VERMA: The only protein that they know about, the cyclosporinate sensitivity, is this large pore, but whether that pore is one single protein or many proteins--that pore hasn't been isolated.

But I do want to comment on some work that Andy and I have been working on in our lab, again dealing with the delta opiate stuff. There's a group, a fellow by the name of Delacey. He exposes mice to 15 minutes of anoxia, and he gets almost 100 percent mortality.

If he gives them five minutes of hypoxic exposure, either once or twice, and then gives them the 15 minutes of anoxia, they survive.

DR. VIRMANI: That's preconditioning.

DR. VERMA: He can block the preconditioning with Meloxone. Furthermore, he can block it with a specific delta opiate antagonist. And his most recent paper, which is the '95 paper, he can induce the tolerance with a delta opiate agonist, rather than with the hypoxia.

DR. VIRMANI: They have also shown adenosine to be important in preconditining - if you give an animal adenosine, at least to the myocardium, you can induce the same thing, i.e. preconditioning like protection.

DR. VERMA: In our own experiments, we've been exposing neurons in culture to hypoxia and have shown a very nice time-dependent increase in this hypoxia-inducible factor. And Andy has also found about the same time course in induction in delta opiate receptors. It's culture drawn. Whether that means anything or not, we'll continue to explore that. Whether that's protective, or not.

COLONEL BELLAMY: Let's try to summarize the session by arriving at some degree of unanimity as to what we should recommend if we were responsible for a research program in the broad area of suspended animation.

It seems to me there are two basic questions that have to be addressed. One has to do with the description of the clinical scenario to which the therapeutic intervention would be applicable. I have strong views regarding this question.

The clinical scenario is clearly somebody who sustains a missile wound, results in exsanguinating hemorrhage and death rather shortly afterwards.

In this scenario we would intervene while the patient was in the process of exsanguinating, with some sort of pharmacological intervention which would then protect the tissues for the impending cardiac arrest. In fact, the intervention would undoubtedly cause cardiac arrest by itself. But the arrest would occur in a state where the organs such as, particularly the critical organs, like the brain and heart, have some degree of protection against the next half hour or hour of lack of any perfusion at all.

Would you want to comment on that, what the scenario would be? Or, Dr. Safar, would you want to comment?

MAJOR BRUTTIG: Sure. I could probably give you a short scenario in terms of what I've seen with the animals, and certainly I have read Dr. Bellamy's literature, which he hasn't referred to. I'm surprised.

But when we see uncontrolled hemorrhage in an animal setting, where we've tried to mimic the human condition, and a penetrating injury specifically, we see pressure dropping for the defined injury that we create. And I should define that. In the case of a tear in the aorta, that's a 5 mm tear in a 45-kilo animal.

In the case of the vena cava, it's a 10 mm tear, and in the case of the femoral artery in the same size animal, the pig, it's a 5 or 6 mm tear, and I can't remember that, particularly.

So these are particularly large tears in the vena cava and the femoral artery, and a reasonably small tear corresponding to diameter in the aorta.

COLONEL BELLAMY: Is that retroperitoneal? I mean, it would have to be--

MAJOR BRUTTIG: Yes.

COLONEL BELLAMY: But is there any possibility of tamponade from retroperitoneal tissue?

MAJOR BRUTTIG: Not really, no. I mean, this bleeds primarily into the abdomen.

COLONEL BELLAMY: There is free bleeding into the abdominal cavity.

MAJOR BRUTTIG: And there can be, if you're not careful in doing it, some retroperitoneal dissection. But no, it goes into the abdomen. If it's a free bleed out of the aorta or the vena cava, the way we set the prep up.

Pressure drops very quickly. Pressure goes from a mean of about a 100 to a mean of 25 in something like a minute or less. You see a rapid pressure drop in the first three cardiac cycles.

And then it's maintained at that level for about five minutes. Following that, there's a very slow but steady rise in pressure over the course of two hours to a mean of 55 mm of mercury. And these days they're published in "Circ Shock."

The data that are published in "Circ Shock" happen to be in a halothane-anesthetized animal. People could argue about the effects of anesthesia, but I can tell you that the same pressure profile is true in the conscious animal. So if he's accomplishing the same thing by a different mechanism, I can't speak to that.

I gave you some indication of what happens in the conscious animal, but it appears that because of the pressure drop, and therefore, the transmural pressures across the vascular wall, that bleeding slows down because an intravascular pressure of 25 and an increasing extravascular, that is, abdominal pressure which goes up to about 18, slows the bleed. Plus any visceral pressure against the wound and the developing thrombus. All of those things take place to slow the bleeding.

The bleeding slows less quickly from the vena cava, pressure is much lower, but it proceeds for a longer period of time.

If you look at the exposed femoral artery, when we caused a bleed in that, it stops in about 8 or 9 minutes. But the pressure profiles are exactly the same. All three of them drop peripheral vascular resistance.

So the animals begin recruiting a number of physiologic mechanisms to overcome that drop in pressure, and most likely--although we haven't looked at it--that flow drops quite a bit, liver flow drops quite a bit. But those have been shown in controlled, fixed-volume hemorrhages, and they've certainly been shown in the Wiggers hemorrhages, the same kinds of decreases in blood flow to the gut.

My guess is that when pressure starts to rise, and certainly within--there's some maintenance of flow to the heart because we don't see any change in EKG in the heart during this--that very quickly flow readjusts in the heart, very quickly flow readjusts to the brain.

And we don't see--although we see a similar change in the lung in terms of pulmonary vascular resistance, we don't see any compromise in terms of--we see a change in extraction. Extraction goes up in order to maintain oxygen consumption. But the animal doesn't appear to be compromised at the lung. That's about all I can tell you. But it's very quick.

COLONEL BELLAMY: That's very interesting. The fact of the matter is, though, people do die of exsanguinating hemorrhage, truncal hemorrhage, so there's a problem here between models and the human clinical experience. Dr. Champion wants to comment. Obviously--

MAJOR BRUTTIG: Could I mention one more thing? People here have referred to the Russian literature and the fact that it's difficult to interpret, and I wish I had a translated copy. All I can tell you is that there is a report from the Russian literature dealing with a particularly gruesome set of experiments on dogs, combining hemorrhage and trauma, and what I've explained to you really is hemorrhage alone.

And any time you impose hemorrhage and trauma together, survival is shortened.

COLONEL BELLAMY: Blast effects as well as hemorrhage?

MAJOR BRUTTIG: What they did was to tear off the hind limb of an animal, which is a survivable trauma. Or they imposed a survivable but massive hemorrhage. But when you do the two together, they don't survive.

So what I'm getting at is that the imposition of trauma, the disruption, the temporary cavitation, or whatever, caused by high velocity missile penetration is probably sufficient to drive you over the edge.

DR. LEWIS: There's another problem with trauma. Trauma to skeletal muscle, gives you a marked vasodilatation, and this is then acting like an arterio-venous fistula, which then may interfere with blood supply to the brain and the heart.

DR. SIESJO: Can I ask, how much do you know about these things, I mean, if we talk about models--I mean, --- [?].

COLONEL BELLAMY: Well, my experience is almost exclusively with a military data base, which has something on the order of twelve hundred who died on the battlefield, and there are extensive autopsy studies, with some description as to how rapidly they died, based on

descriptions by bystanders which are repleats with statements such as the casualty appeared to be dead within five minutes. The corresponding autopsy typically showed several liters of blood within the abdomen or chest.

My impression is that the major cause of death in combat is in fact exsanguinating hemorrhage. The interesting question whether it could be that our therapy, by trying to raise blood pressure with fluids, in fact, simply is terribly bad because all it does is to restart the bleeding.

MAJOR BRUTTIG: And what you see is that some people are now addressing hypotensive resuscitation.

COLONEL BELLAMY: Not to do anything from the standpoint of--

MAJOR BRUTTIG: Or mild resuscitation, for that reason.

COLONEL BELLAMY: Yes. Now, regarding the civilian experience with hemorrhage, there was a study out of San Diego a few months ago which gave hemorrhage as being the cause of death in about 30-odd percent of people who were pronounced dead from trauma in San Diego County a year or two ago.

So it's a common cause it's probably not as important as in the military population.

Maybe Dr. Champion would like to tell--what is a realistic scenario to try to model here?

DR. CHAMPION: Well, we've actually looked at all the deaths in Baltimore from gunshot wounds, I think it was a 1994 data base, and the pattern is very similar to the military one in that about 40 to 50 percent of them died from potentially salvageable things, like gunshot wounds or pneumothorax. There's a lot of similarities in the penetrating wounds in a civilian environment than there is in a military environment.

This study that we did looked at all the medical examiners' cases as well as the ones that got to hospital. So it wasn't sort of limited in the fashion that a lot of others are. It had good autopsy data on all the ones who were never even taken to hospital.

But you know, the scenario you paint, it has got validity when related to the clinical scenario that we've seen in the population here in sunny downtown D.C. over the past 20 years, where you've got quite a number of individuals coming in with exsanguinating hemorrhage. And it's difficult to sort of aggregate the data, but they certainly reach a steady state, with a fairly low blood pressure. They're usually very vasoconstricted, and that's why I was wondering what happened to the systemic vascular resistance in this.

MAJOR BRUTTIG: In all three cases, the vascular resistance goes down and stays down for a prolonged period of time. You couldn't argue it solely on the basis of the hole in the vessel.

DR. CHAMPION: Yes. That's why I can't--that's the bit that doesn't fit in, and I can't, you know, relate that piece at all.

MAJOR BRUTTIG: Well, let me give you one more piece of evidence. We've looked at the micro circulation, and in skeletal muscle, the micro circulation does constrict for a bit. And then after about 10 minutes, there's an escape from that to almost control of flow.

So it's a muddier picture there. You would expect that you'd see the micro circulation dilate. That would be fully supporting the idea of a decrease in peripheral vascular resistance.

DR. CHAMPION: What happens to the heart rate during this period of time? Does that follow the sort of expected clinical course of this sort of significant tachycardia, and then sort of--

MAJOR BRUTTIG: No, no. In fact, if we see a very significant tachycardia, we know the animal's going to die. What we see is no change in heart rate for about 30 minutes, and then a slow drift up to about 140 or 150.

DR. CHAMPION: I think there's not a homogenous population. I mean, there's probably various populations. If you take the clinical scenarios that we're presented with, there's probably a number of different patient populations, some of whom are going to die no matter what you do, some of whom are going to die because we start interfering with them, replacing their blood with water, as Maddox has been saying, and sort of screwing up all of these homeostatic mechanisms and coagulations mechanisms.

And then there's the group who will probably survive, no matter what we do to them, and those are the ones we'll probably save.

DR. VIRMANI: You don't want to take the credit.

DR. CHAMPION: It's a question of extending that patient population--well, A, teasing it out, and I think, you know, there's various data bases we can do that on. You know, the WOMET one is one, and NTS data base, I think we've got 175,000 patients in that, and we were going to do a sort of a case control methodology, looking at people who died of exsanguinating hemorrhage versus those who didn't, and sort of map those on a time basis, and we could probably do that analysis, and try and tease out some of these factors, and look at some of the elements that differentiate, go down a layer or two in terms of the animals and differentiate those that die and those that don't die, and tease out some of these differences, and that might help a little bit, configure the scenario with a little bit more precision.

COLONEL BELLAMY: There are at least some casualties, perhaps a substantial number, who would have unrecognized vascular injuries. I think Norm Rick's data from the Vietnam War indicated that this entire vascular registry did happen. But I can't imagine it being particularly common that you're going to have people who have substantial aortic or inferior vena cava laceration which are unrecognized. I just don't--

DR. CHAMPION: You know, sometimes the injuries--you know, I've certainly seen them with fairly discrete injuries, you know, but discrete like through and through stab wounds in the inferior vena cava, where, you know, a trained but youngish surgeon has not recognized the injury and he's about to close the abdomen.

I mean, to that degree of lack of recognition, where there has been a hematoma, a certain amount of tamponade, and clots, and the natural history of that might be very benign--I don't know--but nevertheless, you know, these things can happen.

COLONEL BELLAMY: Let's ask Dr. Safar what he thinks about a realistic clinical scenario.

DR. SAFAR: I don't think we can and should limit ourselves to one scenario. As we are taking about models in existence now, it's so model-dependent as to what happens. Exactly the same blood pressure course you described, we have in our unanesthetized, volume-controlled hemorrhagic shock model in rats. What happened after the aortic tear? Sometime will it close off?

You then have the whole organism in low flow, with viscera closing off completely at times. When you finally produce hemostasis and resuscitation, you get temporary recovery. In two days, multiple organ failure occurs. That is what you want to prevent.

I think this group should decidenot only on one scenario., Let us say that we know or suspect certain limits of low-flow or no-flow, which the organism can tolerate. How can we extend it? Let us then decide whether we want to limit ourselves to a rapidly or slowly developed cardiac arrest situation of X minutes or hours, or whether we want to go also into uncontrolled hemorrhagic shock state treated with the hibernation approach. The former has more to do with suspended animation. The two belong together in a fashion, if we want to really do the best for the most.

COLONEL BELLAMY: I think that's very valid--

COLONEL BELLAMY: Okay. So there would be at least two models, two different clinical scenarios that would need to be considered by the Navy in its program.

DR. CHAMPION: But one of the important things I think is the systemic vascular resistance. I mean, if you

look at the spinal cord injury, you know, the low-flow, low-resistance state is very protective of--

COLONEL BELLAMY: Oh, yes, of course.

DR. CHAMPION: And, you know, that makes sense, that people would--you know, as a form of protection, the vascular resistance would go down. You know, if that's to be part of the model, that should be very explicit, because it's so protective, I think.

COLONEL BELLAMY: You could argue that the thing to do is induce a state of low blood pressure by some vasodilator.

DR. CHAMPION: Right, yes.

COLONEL BELLAMY: Yes?

T7 DR. SIESJO: I agree with Peter. The problem must be to tackle the problem using several models, because with some models you can study individual organs, like the heart or the brain, and you can look at such things as reperfusion damage, what is it due to. Is it mitochondrial failure, which comes later on? Is it microvascular failure?

But then you bring the whole thing up to a much more complicated level where you look at the integrated systems in the whole body, because then you have the problem of multi-organ failure and all that.

DR. SAFAR: It will depend on how much money you have and how many groups you want to support. I can see a systematic approach to the whole thing. We do not have time now. I was thinking about four groups of questions to attack with a multi-level research approach, from the molecular, cellular, to organ, organ system and organism level. Then you can go from rats to large animals, and finally to people.

While this is going on, there may be some other parallel approach going on, where a less systematic and more serendipitous approach is used. For example, we should reexplore pharmacologic hibernation attempts of the earlier years, which really have not been studied well then when there was no reliable life support.

In other words, how much can one simulate what certain animals do with certain existing pharmaco--without going into anything magic. Like overdose with anesthetics. You can inject the volatile anesthetic through the trachea. A medic could do that. Overdosing. If this would do it. We don't know.

MAJOR BRUTTIG: One of the things I think you have to be real careful of is that for people that don't understand hibernation, they can easily be tricked by what's out in the literature. And John Hallenbeck's got a nice description of hibernation and a whole series of figures on physiologic performance as opposed to the use of a quithisson, and I don't know--

COLONEL BELLAMY: What is that?

MAJOR BRUTTIG: A quithisson is primarily chloryl hydrate with other things, and it was used to indicate pharmacologic hibernation. Okay. And when you lay the physiologic performance side by side, there's true hibernation, and then there's sedation, and it changed heart rate a little better. You know.

What you really want to understand is what you want to accomplish.

DR. SAFAR: Yes, sure.

MAJOR BRUTTIG: And then choose the method that will really accomplish that for you. My caveat is that the closer you can come to a harmonious approach, I think the fewer drugs you're going to have to put in for the individual symptomologic--

DR. SAFAR: In the protracted low-flow or trickle-flow state of uncontrolled hemorrhagic shock, where you can prevent cardiac arrest from occurring, the target organ which will fail later most likely will be the viscera.

MAJOR BRUTTIG: Yes.

DR. SAFAR: The brain will survive with between 30 and 40 mean perfusion pressure. When you have cardiac arrest, it is the other way around. The brain is the most vulnerable organ.

Do we want to model both? I think, yes.

COMMANDER YAFFE: On the battlefield, in one sense, the scenario problem will be you won't know whether you have this low flow and whether you should immediately do something for resuscitation, or whether you should pickle--

DR. SAFAR: As long as you have, probably, a viable brain blood flow going on, he will still be gasping. You don't need a fancy monitor.

COMMANDER YAFFE: So you think this will be an adequate sign?

COLONEL BELLAMY: Well, ARPA is producing monitors but whether they will work or not, I don't know.

DR. CHAMPION: Well, when they're set for juice, they're measuring temperature so far.

[Laughter.]

COMMANDER YAFFE: And whether there will be clear, sort of, brain points, or whether there's really some spectrum, and whether, as you say, that ultimately what you want to do is put all of those people in--

DR. SAFAR: When he is still gasping, and you think he is doomed, and the only thing you have available is to pickle him, it will stop the heart. You will think twice to

do that. Is there something you have for suspended animation which won't stop the heart? That is the magic thing to look for.

COLONEL BELLAMY: I think, though, the worst-case scenario has to be considered, which is exsanguination, cardiac arrest, and then the question--your paradigm of protection, preservation, and resuscitation comes into play, and the obvious question is, How do you induce protection?

That is, giving some sort of pharmacological substance prior to the onset of cardiac arrest, which, when it occurs, will in fact give some prolonged viability to the heart and brain.

DR. SAFAR: I have a suggestion here which may also make this topic clinically, and socioeconomically, more important than it seems. We are thinking about a small fraction of combat casualties. But -- suspended animation could be a totally new approach to resuscitation in general, also of sudden death cases in the civilian sector.

COLONEL BELLAMY: Yes. Tell us, please.

DR. SAFAR: What we are now doing in the unwitnessed cardiac arrest, the patient found by the medics pulseless, definitely after ? then 5 or 10 minutes of pulselessness, may be the wrong thing.

If somebody drops dead in front of you, and you go rapidly through the A, B, Cs and to defibrillation, that makes sense. And he probably will wake up within an hour, or earlier.

But if you find someone dead, and you do not know how long this arrest has lasted, to jump on him with the frantic application of steps A, B, C of CPR, may be the wrong thing to do. You are now superimposing trickle flow under normothermia. God knows what you're doing with this.

So why not have ready for the medic an iced magic solution, cannulate the carotid artery, flush it with a large volume, and transport him dead. Then go into something new, still to be worked out. Cannulate vessels for artificial circulation and apply a totally newly designed way of bringing life back, without stirring up re-oxygenation injury cascades.

COLONEL BELLAMY: We're open to suggestions.

DR. SAFAR: There must be a way to do it by controlling the temperature and by using pharmaca in a balanced mix.

COLONEL BELLAMY: Well, it's obvious that that clinical population could serve as a--I don't want to say "experimental model"--but at least a clinical trial or something. Then it could be applied in trauma cases. I think it's very--

DR. SAFAR: What I want to say is that this whole suspended animation idea here might have quite a bearing,

which we don't even realize now, on the whole resuscitation field.

COLONEL BELLAMY: Oh, I agree with you. That's why I say the--

DR. SAFAR: Not on just exsanguination.

COLONEL BELLAMY: I have framed a clinical scenario which is very discrete and very military oriented. I'm quite certain that if this sort of approach could be actually brought to fruition, it would find far more use in civilian medicine, particularly in cardiac arrest, and maybe even clinically in a hospital's operations, and so on.

The military model here is simply our justification, because the military is initially going to pay for this sort of thing. But I think the clinical value would be far greater, to extend well beyond military application.

Well, again, the question would be, though, the phases for the most extreme scenario, that is, cardiac arrest, the protection phase, and then the phase of preservation, which obviously would have to be induced, initially, during the protection phase. Resuscitation, it seems to me, is fairly straightforward. There's a vast amount of clinical experience with that, and I'm sure by the time we ever reach the point where clinical trials will be posssibly, we will know far more about preventing multiple organ failure.

So the question really seems to be the direction of the research effort. I would think it would have to be directed toward the initial protection what happens during the first five minutes; the fact that the ionic balance across a brain cell membrane is now in equilibrium is extraordinarily bad.

DR. SIESJO: Well, at least in the rat. I'm not quite sure about man. But I think this is--the scenario should be an ideal one for pre-treatment of what is going to happen. And there are sound pharmacological principles which have proven protective under those circumstances.

And you could use soluble free radical scavenger. You can use a drug which will drop body temperature by 3 to 4 degrees. There are many such drugs.

COLONEL BELLAMY: Like what?

DR. SIESJO: I have a list of them at home. I mean, chlorpromazine is one, and there are some serotonin antagonists, and dopamine, whether agonist, antagonist. But I would be happy to--

COLONEL BELLAMY: That's very interesting. Even as Dr. Safar has shown us, a small fall in temperature can be associated with increased survival.

DR. SIESJO: Yes, exactly. And the Miami group has shown that although NMDA antagonists do not protect

against global or forebrain ischemia with recirculation, they do protect if you combine with them a 3 to 4 degree lowering of body temperature. And there is an NMDA antagonist which are probably circulating also in the armed forces here. That's dextorphin. And dextorphin you can give--I mean, it's an over-the-counter kind of anti-cough medicine. You can combine that with moderate hypothermia and then give a soluble free radical scavenger.

You have a mixture which potentially should prolong--

DR. SAFAR: Going back to the experiences of the 1950s when therapeutic hypothermia was induced with any anesthetics, there are certain differences in the rate by which core temperature would go down. But any anesthetic will enhance the drop in core temperature and brain temperature if you just expose the person to environmental temperature--

COLONEL BELLAMY: Remember, the United States Army always fights in climates like Washington.

DR. BENTLEY: That's one problem I see with treating somebody with a drug that depresses metabolism. I think those drugs you were mentioning might lower metabolic rate, but it's going to take quite a long time to cool a person off. They're a big bulk of water, and it's going to take maybe half an hour, an hour, to actually cool them down, chill then off, and so on.

DR. SAFAR: You need more than that.

MAJOR BRUTTIG: In addition, I should point out that my feeling is that we're not going to identify a magic bullet, and so if we can do a number of things, which, in concert, accomplish what it is that we're trying to achieve, and be able to control the situation, we're probably going to be better off, at least initially, until somebody can refine the magic bullet.

My experience is, and the commissions can correct me--oftentimes, when you induce shock and you get a peripheral constriction, you may in fact get some cooling of a degree or so anyhow--body cooling.

If you then, pharmacologically, attempt to drop body temperature a little bit, that's a good thing. Currently under development out in DOE labs, in Washington, they're working on microdevice and nanodevice. They're essentially molecular constructs.

The idea is that you can paint your windows with this stuff and air condition your house because they're a tremendous heat sink.

And we're picking up on that in the Army, to put inside of these advanced critical care litters, to cool the patient mildly.

COLONEL BELLAMY: I'm sorry. Repeat. What is this substance?

MAJOR BRUTTIG: There are a series of what they call micro- and nanodevices. They're molecular devices.

COLONEL BELLAMY: Which do what?

MAJOR BRUTTIG: Pardon?

COLONEL BELLAMY: Which do what?

MAJOR BRUTTIG: A variety of things. Pumping. Heat sinks. Countercurrent heat exchangers. But since we're talking about cooling, that's why I thought this might be a particularly apropos kind of treatment. And then there are pads that can be made from a fiberfill with a water-cooling device.

Now, imagine, if you will, if you put somebody in an enclosed chamber--and bear with me for a minute because we're talking about far forward on the battlefield. You're not going to have enclosed chambers that far forward on the battlefield, but there are some ways to get around that.

If you put somebody in an enclosed chamber, and their tendency is to cool anyhow, and you blow over them with a fan, and mild cooling environment, you can drop them down to 3 or 4 degrees, because they have no way to overcome that, since their blood pressure is down, and their peripheral perfusion is down, and things like that. So you probably can get them down.

I would worry that you're going to drive them too far down, and so we have to be able to warm them and keep them where we want to keep them, too.

COLONEL BELLAMY: Yes, Dr. Bazan?

DR. BAZAN: One comment, coming back to the initial injury mechanisms, and neuroprotection, addressing the question that you're posing, Colonel Bellamy, on neuroprotection, I think presynaptic release of excitotoxin precedes lipid peroxidation, precedes glutaminase receptor activation, precedes all of that, and from the work that our labs have done about 10 years ago, three new generations of PAF antagonists have been developed, and there are 20 labs in the world now very actively also looking, for example, at synaptic tegmen [?], and synaptic-specific proteins.

I don't believe, as we have discussed with John Hallenbeck and Bo Siesjo during the break, I don't believe that this is the only player, PAF, in the presynaptic mechanisms. But I think this gives us some new clues to go and try to slow down the initial insult that triggered brain damage.

Perhaps that's one idea that could be listed in the thinking.

COLONEL BELLAMY: Right. Dr. Lewis.

DR. LEWIS: If I understand Dr. Safar correctly, what you're saying is the individual is dead. Their heart has stopped, respiration has stopped, there's no blood flow.

DR. SAFAR: That's one scenario.

DR. LEWIS: Yes. And all of us are under the assumption that when that has happened and a certain period of time has gone, the changes are irreversible.

And what you're suggesting is that maybe they're not. Maybe they're not irreversible. Maybe the way we're approaching it is to make them irreversible. And if this is the case, I think this is extremely interesting because this could apply to all scenarios; namely, that what we're doing is killing the people rather than saving them in this way. This is certainly one approach that might give some very interesting benefit, because what that means is that maybe the essential feature is not that you must do something immediately. Maybe the essential feature is don't do anything until you can get into a quiet place where you can start doing things that are the right approach--

COLONEL BELLAMY: I don't think anybody thinks it's reasonable to do nothing for somebody who has a cardiac arrest right here

DR. LEWIS: That's not what I'm talking about.

COLONEL BELLAMY: You're talking about--

DR. LEWIS: I agree with him. If you're there when the cardiac arrest happens, of course you don't wait. But if it happens and you're not there, maybe all this, as you say, panic that you're doing, is wrong.

COLONEL BELLAMY: Yes; we'll think about that.

COMMANDER YAFFE: But there would be a different set of things to do to prevent further deteriorations.

DR. LEWIS: Maybe.

DR. SIESJO: David, you're absolutely right. But I mean, the thing we probably have missed for many, many years is the fact that you may be seriously ischemic for a very long time and still achieve salvage of the tissue, because what you are experiencing is reperfusion damage.

We are at a stage now when people have been able to occlude the middle cerebral artery distally, so you can an infarct of a certain size, and by a combination treatment, which is directed against apoptosis and against NMDA receptors, it has been possible to reduce this damage to 15 percent of what it was without treatment. Now, that's 90 minutes of ischemia. This is pretreatment.

DR. CHAMPION: So the first thing to do would be to stop reperfusion injury and then go on with the treatment.

COLONEL BELLAMY: Reperfusion is required to vital organs, ultimately. You have to have some blood flow sooner or later, I think.

Dr. Safar, I think, had a comment.

DR. SAFAR: Merely on the name of our group. You say our group would be abandoned, now so the name doesn't matter anymore. Neither Lazarus nor Pharmacologic--

COLONEL BELLAMY: Well, Lazarus is considered inflammatory, so--

DR. SAFAR: The discussion really has shown that we are talking about more than pharmacology.

COLONEL BELLAMY: So Dr. Safar does not like the idea of the word in the title, pharmacological.

DR. SAFAR: Not to limit it to this.

COLONEL BELLAMY: Well, of course, if we accept a variety of scenarios, clearly it goes beyond pharmacological. Although I still think, though, with the most extreme scenario, the approach initially must be pharmacological. Later on, of course, hypothermia and other--cardiopulmonary bypass would be an integral part of it.

But the question would be whether or not changing the word pharmacological to, say, what? Surgical stabilization?

MAJOR BRUTTIG: Intervention.

DR. SAFAR: Do you feel that the words "suspended animation" would scare--

COLONEL BELLAMY: No. Well, in fact--

COMMANDER YAFFE: I would use "life sustainment."

DR. SAFAR: Suspended animation for delayed resuscitation.

COLONEL BELLAMY: Well, on that note, we'll end our meeting.

[Whereupon, at 4:57 p.m., the meeting was adjourned.]